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(54) Title: ENVIRONMENTAL STRESS TOLERANCE GENES

(57) Abstract: Recombinant polynucleotides and methods for modifying the phenotype of a plant are provided. In particular, the phenotype that is being modified is a plant's environmental stress tolerance.

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ENVIRONMENTAL STRESS TOLERANCE GENES**RELATED APPLICATION INFORMATION**

5 The present invention claims the benefit from US Provisional Patent Application Serial Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait Modification III" filed August 22, 2000.

FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

BACKGROUND OF THE INVENTION

10 Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

15 Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics.

20 Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

25 The present invention provides novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's environmental stress tolerance.

SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a

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complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's environmental stress tolerance; (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above. In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention may be a plant lacking a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27.

The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having
5 improved environmental stress tolerance. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified environmental stress tolerance.

In another aspect, the invention relates to a method of identifying a factor that is
10 modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional
15 protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method
20 comprises placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a
25 polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant environmental stress tolerance phenotype.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides
30 encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

10 Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

15 Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

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DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

25 In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance, such as freezing, chilling, heat, drought, water saturation, salt, photoconditions, radiation and ozone, or the like. Plants with altered expression of the
30 polynucleotides or polypeptides of the invention are more tolerant to these environmental stresses compared with plants without altered expression levels.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one

or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e., expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

DEFINITIONS

A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation

site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under

regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic

is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural
5 observations such as stress tolerance, yield or pathogen tolerance.

“Trait modification” refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or
10 decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution
15 observed in wild type plant.

Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved
20 tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of
25 taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenillipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition.

Physical plant characteristics that can be modified include cell development (such as the number
30 of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time,

flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

5 The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's environmental stress tolerance.

10 Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

15 Additional polynucleotides of the invention were identified by screening *Arabidopsis thaliana* and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the
20 manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

25 The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the environmental stress tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the environmental stress resistance of plants.

Making polynucleotides

30 The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or

single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

A variety of methods exist for producing the polynucleotides of the invention.

- 10 Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology,
15 F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

- Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through
20 in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis).
25 Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR
30 expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, *all supra*.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically

- ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

HOMOLOGOUS SEQUENCES

- Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from *Arabidopsis thaliana* or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such as pine, poplar and eucalyptus.

- Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the

listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65°C, for example 0.2 x SSC, 0.1% SDS at 65°C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50°C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique

coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

SEQUENCE VARIATIONS

It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

Amino acids			Codon							
Alanine	Ala	A	GCA	GCC	GCG	GCU				
Cysteine	Cys	C	TGC	TGT						
Aspartic acid	Asp	D	GAC	GAT						
Glutamic acid	Glu	E	GAA	GAG						
Phenylalanine	Phe	F	TTC	TTT						
Glycine	Gly	G	GGA	GGC	GGG	GGT				
Histidine	His	H	CAC	CAT						
Isoleucine	Ile	I	ATA	ATC	ATT					
Lysine	Lys	K	AAA	AAG						
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT		
Methionine	Met	M	ATG							
Asparagine	Asn	N	AAC	AAT						
Proline	Pro	P	CCA	CCC	CCG	CCT				
Glutamine	Gln	Q	CAA	CAG						
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT		
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT		
Threonine	Thr	T	ACA	ACC	ACG	ACT				
Valine	Val	V	GTA	GTC	GTG	GTT				
Tryptophan	Trp	W	TGG							
Tyrosine	Tyr	Y	TAC	TAT						

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

- 5 Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

10

Table 2

Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser; Val
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of

the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

10 FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51: 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

25 EXPRESSION AND MODIFICATION OF POLYPEPTIDES

Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or

developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the

- 5 TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (*see, e.g., Odel et al. (1985) Nature 313:810*); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

- 10 A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known
15 promoters have been characterized and can favorably be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the *dru 1* promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No.
20 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol
25 Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et
30 al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea *rbcS-3A* promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize *rbcS* promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., *wun1*, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-

396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development (Odell et al. (1994) Plant Physiol 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Additional Expression Elements

Specific initiation signals can aid in efficient translation of coding sequences.

These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

Expression Hosts

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e., nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., 5 (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., 10 (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of *Agrobacterium tumefaciens* or *A. rhizogenes* carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, 15 wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression 20 can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors 25 containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

Modified Amino Acids

Polypeptides of the invention may contain one or more modified amino acids. 30 The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References
5 adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

IDENTIFICATION OF ADDITIONAL FACTORS

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phenotype or trait of
10 interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene
15 with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g., a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional
20 gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific
25 nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al.
30 (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any

method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions *in vivo* and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

20 IDENTIFICATION OF MODULATORS

In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northern, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified

molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) *Nature Biotechnology*, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. *Science* (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum *C&EN* Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton et al. *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems
5 utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Indeed, entire high throughput screening systems are commercially available.
10 These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

15 The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators
20 that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with
25 cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any
30 additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,

plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

SUBSEQUENCES

Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

PRODUCTION OF TRANSGENIC PLANTS

Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the environmental stress resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved environmental stress tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably,

the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of
5 RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No.
10 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor
15 homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with
20 antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to
25 suppress expression of an endogenous transcription factor, thereby reducing or eliminating its activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

30 Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion

event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean,

clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture –Crop Species.

- 5 Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such improved environmental stress tolerance, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., *supra*.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is

referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for
5 nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of
10 one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E)
15 of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided
20 by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or
25 even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface
30 allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular

phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single
5 computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or
10 homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

15 Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a
20 centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

25 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor
30 sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, *Arabidopsis thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60°C) and labeled with ³²P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO₄ pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSSC, 1% SDS at 60°C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon™ cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon™ Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into

competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini
5 Prep kits (Qiagen, CA).

EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of
10 *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A_{600}) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were
15 centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

Agrobacterium cells were transformed with plasmids prepared as described
20 above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were
25 immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to

transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 μ E/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

20 EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H₂O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H₂O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 μ E/m²/sec) at 22-23°

C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T_1 generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

5 Primary transformants were crossed and progeny seeds (T_2) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

10

EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

15 The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) *Plant Cell* 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 base pairs to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by
20 PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and
25 Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

EXAMPLE VII. IDENTIFICATION OF ENVIRONMENTAL STRESS TOLERANCE PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

30 Experiments were performed to identify those transformants or knockouts that exhibited an improved environmental stress tolerance. For such studies, the transformants were exposed to a variety of environmental stresses. Plants were exposed to chilling stress (6 hour exposure to 4-8° C), heat stress (6 hour exposure to 32-37° C), high salt stress (6 hour exposure to 200 mM NaCl), drought stress (168 hours after removing water from trays), osmotic stress (6 hour exposure to 3 M mannitol), or nutrient limitation (nitrogen, phosphate, and potassium) (Nitrogen: all components of MS medium remained constant except N was reduced to 20mg/L of

NH₄NO₃, or Phosphate: All components of MS medium except KH₂PO₄, which was replaced by K₂SO₄, Potassium: All components of MS medium except removal of KNO₃ and KH₂PO₄, which were replaced by NaH₄PO₄).

5 Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

Table 3

SEQ ID No.	GID	Knockout (KO) or overexpressor (OX)	Phenotype observed
1	G22	OE	Increased tolerance to high salt
3	G188	KO	Better germination under osmotic stress
5	G225	OE	Increased tolerance to nitrogen-limited medium
7	G226	OE	Increased tolerance to nitrogen-limited medium
9	G256	OE	Better germination and growth in cold
11	G419	OE	Increased tolerance to potassium-free medium
13	G464	OE	Better germination and growth in heat
15	G482	OE	Increased tolerance to high salt
17	G502	KO	Increased sensitivity to osmotic stress
19	G526	OE	Increased sensitivity to osmotic stress
21	G545	OE	Susceptible to high salt
23	G561	OE	Increased tolerance to potassium-free medium
25	G664	OE	Better germination and growth in cold
27	G682	OE	Better germination and growth in heat
29	G911	OE	Increased growth on potassium-free medium
31	G964	OE	Better germination and growth in heat
33	G394	OE	More sensitive to chilling
35	G489	OE	Increased tolerance to osmotic stress

10 For a particular overexpressor that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with an increased expression of the particular transcription factor.

EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

15 Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the

BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6×10^{-40} is 3.6×10^{-40} . For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-*Arabidopsis* genes shown in Figure 3 and the *Arabidopsis* genes in the sequence listing are: SEQ ID No. 1: 53%-67%; SEQ ID No. 3: 38%-76%; SEQ ID No. 5: 34%-67%; SEQ ID No. 7: 50%-69%; SEQ ID No. 9: 32%-91%; SEQ ID No. 11: 48%-66%; SEQ ID No. 13: 34%-60%; SEQ ID No. 15: 58%-81%; SEQ ID No. 17: 65%-94%; SEQ ID No. 19: 72%-83%; SEQ ID No. 21: 52%-64%; SEQ ID No. 23: 40%-89%; SEQ ID No. 25: 86%-97%; SEQ ID No. 27: 41%-75%; SEQ ID No. 29: 29%-72%; SEQ ID No. 31: 49%-70%; SEQ ID No. 33: 56%-86%; SEQ ID No. 35: 61%-84%; SEQ ID No. 37: 40%-58%; SEQ ID No. 39: 63%-87%; SEQ ID No. 41: 51%-88%; SEQ ID No. 43: 80%-90%; SEQ ID No. 45: 79%-90%; SEQ ID No. 47: 30%-58%; SEQ ID No. 49: 52%-62%; SEQ ID No. 51: 55%-73% and SEQ ID No. 53: 44%-80%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the environmental stress tolerance of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with

reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

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What is claimed is:

1. A transgenic plant with modified environmental stress tolerance, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - 5 (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;
 - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
 - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a complementary nucleotide sequence thereof;
 - 10 (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
 - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
 - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
 - 15 (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
 - (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g);
 - 20 (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
 - (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
 - 25 (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and
 - (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.
2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

5

4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- 15 (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which
- 20 subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
- (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g);
- (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide
- 25 sequence of any of (a)-(g);
- (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
- (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and
- 30 (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.

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5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.
6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.
7. A cell comprising the cloning or expression vector of claim 6.
8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
9. A composition produced by one or more of:
(a) incubating one or more polynucleotide of claim 4 with a nuclease;
(b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
(c) incubating one or more polynucleotide of claim 4 with a polymerase;
(d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
(e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
(f) incubating one or more polynucleotide of claim 4 with a cell.
10. A composition comprising two or more different polynucleotides of claim 4.
11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
12. A plant ectopically expressing an isolated polypeptide of claim 11.
13. A method for producing a plant having a modified environmental stress tolerance, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for improved environmental stress tolerance thereby providing the modified plant with a modified environmental stress tolerance.
14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

5

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:

- 15 (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
- (b) monitoring one or more of:
 - (i) expression level of the polynucleotide in the plant;
 - (ii) expression level of the polypeptide in the plant;
 - 20 (iii) modulation of an activity of the polypeptide in the plant; or
 - (iv) modulation of an activity of the polynucleotide in the plant.

19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.

25

20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant environmental stress tolerance phenotype.

30

21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:

- (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

5

22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

10

23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

15

24. The method of claim 21, further comprising linking the one or more of the polynucleotides of claim 4, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.

20

25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

25

27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G22	cDNA	
2	G22	protein	89-157
3	G188	cDNA	
4	G188	protein	175-222
5	G225	cDNA	
6	G225	protein	39-76
7	G226	cDNA	
8	G226	protein	28-78
9	G256	cDNA	
10	G256	protein	13-115
11	G419	cDNA	
12	G419	protein	392-452
13	G464	cDNA	
14	G464	protein	7-15,70-80,125-158,183-219
15	G482	cDNA	
16	G482	protein	25-116
17	G502	cDNA	
18	G502	protein	10-155
19	G526	cDNA	
20	G526	protein	21-149
21	G545	cDNA	
22	G545	protein	82-102, 136-154
23	G561	cDNA	
24	G561	protein	248-308
25	G664	cDNA	
26	G664	protein	13-116
27	G682	cDNA	
28	G682	protein	22-53
29	G911	cDNA	
30	G911	protein	86-129
31	G964	cDNA	
32	G964	protein	126-186
33	G394	cDNA	
34	G394	protein	121-182
35	G489	cDNA	
36	G489	protein	57-156

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
37	G463	homolog of G464	cDNA	14-23, 77-88, 130-146, 194-227
38	G463	homolog of G464	protein	
39	G767	homolog of G502	cDNA	8-158
40	G767	homolog of G502	protein	
41	G765	homolog of G526	cDNA	23-167
42	G765	homolog of G526	protein	
43	G197	homolog of G664	cDNA	14-119
44	G197	homolog of G664	protein	
45	G255	homolog of G664	cDNA	14-115
46	G255	homolog of G664	protein	
47	G1113	homolog of G911	cDNA	85-128
48	G1113	homolog of G911	protein	
49	G398	homolog of G964	cDNA	128-191
50	G398	homolog of G964	protein	
51	G395	homolog of G394	cDNA	72-135
52	G395	homolog of G394	protein	
53	G393	homolog of G394	cDNA	106-169
54	G393	homolog of G394	protein	

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G22	790359	1.00E-45	Nicotiana tabacum
1	G22	3342210	6.60E-45	Lycopersicon esculentum
1	G22	6654776	1.60E-44	Medicago truncatula
1	G22	8809570	5.80E-44	Nicotiana glauca
1	G22	7627061	2.40E-39	Gossypium arboreum
1	G22	7324479	9.50E-36	Lycopersicon pennellii
1	G22	8980312	4.30E-31	Catharanthus roseus
1	G22	7528275	1.20E-30	Mesembryanthemum crystallinum
1	G22	6478844	4.60E-28	Matricaria chamomilla
1	G22	6847348	5.90E-26	Glycine max
3	G188	7779802	5.20E-36	Lotus japonicus
3	G188	7284340	2.10E-34	Glycine max
3	G188	9361307	1.20E-27	Triticum aestivum
3	G188	7340336	1.10E-22	Oryza sativa
3	G188	6529152	3.60E-22	Lycopersicon esculentum
3	G188	8748477	7.70E-21	Medicago truncatula
3	G188	5456433	7.10E-14	Zea mays
3	G188	9302479	1.60E-12	Sorghum bicolor
3	G188	6696287	4.10E-12	Pinus taeda
3	G188	562242	9.00E-12	Brassica rapa
5	G225	4396287	4.40E-16	Glycine max
5	G225	309571	0.00029	Zea mays
5	G225	3857004	0.001	Populus tremula x Populus tremuloides
5	G225	9410205	0.019	Triticum aestivum
5	G225	9426190	0.025	Triticum turgidum subsp. durum
5	G225	8382118	0.046	Gossypium arboreum
5	G225	6782756	0.27	Oryza sativa
5	G225	7721017	0.4	Lotus japonicus
5	G225	6020136	0.47	Pinus taeda
5	G225	2921331	0.48	Gossypium hirsutum
7	G226	4396287	5.10E-15	Glycine max
7	G226	9410205	1.50E-05	Triticum aestivum
7	G226	3857004	0.11	Populus tremula x Populus tremuloides
7	G226	2428139	0.35	Oryza sativa
9	G256	1430847	1.30E-72	Lycopersicon esculentum
9	G256	9252441	1.20E-65	Solanum tuberosum
9	G256	8380712	2.20E-58	Gossypium arboreum
9	G256	8172976	1.60E-54	Medicago truncatula
9	G256	9205295	1.30E-44	Glycine max
9	G256	20562	6.40E-40	Petunia x hybrida
9	G256	4886263	4.40E-37	Antirrhinum majus
9	G256	6552360	5.00E-36	Nicotiana tabacum
9	G256	2312003	1.20E-35	Oryza sativa
9	G256	5268628	5.20E-35	Zea mays
11	G419	7239156	2.60E-59	Malus x domestica
11	G419	5278451	9.00E-58	Lycopersicon esculentum
11	G419	9205496	1.30E-55	Glycine max
11	G419	7628137	9.30E-51	Gossypium arboreum
11	G419	6069643	9.50E-51	Oryza sativa
11	G419	7562931	9.80E-45	Medicago truncatula
11	G419	7322293	2.30E-37	Lycopersicon hirsutum
11	G419	8404716	1.10E-29	Hordeum vulgare
11	G419	7217755	1.40E-29	Sorghum bicolor

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G419	9428023	4.60E-28	Triticum aestivum
13	G464	6527230	3.60E-31	Lycopersicon esculentum
13	G464	9305572	1.10E-22	Sorghum bicolor
13	G464	6604917	6.70E-22	Medicago truncatula
13	G464	5058123	2.30E-21	Glycine max
13	G464	3760881	1.20E-19	Oryza sativa
13	G464	5044476	1.20E-17	Gossypium hirsutum
13	G464	9412603	6.40E-15	Triticum aestivum
13	G464	7777277	3.20E-13	Lotus japonicus
13	G464	9410371	1.70E-11	Hordeum vulgare
13	G464	7624108	2.10E-10	Gossypium arboreum
15	G482	7691987	5.50E-50	Glycine max
15	G482	7781090	1.30E-48	Lotus japonicus
15	G482	7409616	1.10E-47	Lycopersicon esculentum
15	G482	9416562	4.40E-46	Triticum aestivum
15	G482	22379	2.30E-44	Zea mays
15	G482	7501372	7.70E-44	Gossypium arboreum
15	G482	7765436	8.40E-42	Medicago truncatula
15	G482	5044464	1.20E-40	Gossypium hirsutum
15	G482	9441376	9.20E-40	Chlamydomonas reinhardtii
15	G482	8071558	3.50E-39	Solanum tuberosum
17	G502	6730941	1.60E-91	Oryza sativa
17	G502	7765679	1.60E-82	Medicago truncatula
17	G502	7502501	7.30E-80	Gossypium arboreum
17	G502	5510359	8.30E-77	Glycine max
17	G502	5601137	8.70E-76	Lycopersicon esculentum
17	G502	9302206	1.40E-73	Sorghum bicolor
17	G502	4089948	3.40E-50	Brassica napus
17	G502	8329134	7.90E-49	Mesembryanthemum crystallinum
17	G502	7723564	8.60E-49	Lotus japonicus
17	G502	4218534	1.80E-48	Triticum sp.
19	G526	5049217	3.40E-61	Gossypium hirsutum
19	G526	6066594	1.50E-55	Petunia x hybrida
19	G526	4384535	1.50E-54	Lycopersicon esculentum
19	G526	6454868	6.60E-54	Glycine max
19	G526	4977542	4.70E-52	Oryza sativa
19	G526	5343151	7.00E-51	Zea mays
19	G526	9361647	5.10E-50	Triticum aestivum
19	G526	6799764	4.30E-48	Medicago truncatula
19	G526	8708684	1.80E-47	Hordeum vulgare
19	G526	4218536	3.60E-47	Triticum sp.
21	G545	4666359	8.30E-55	Oenothera glomerata
21	G545	7228328	3.70E-52	Medicago sativa
21	G545	1763062	1.30E-51	Glycine max
21	G545	7206360	3.10E-44	Medicago truncatula
21	G545	7626808	9.60E-40	Gossypium arboreum
21	G545	439492	3.90E-39	Petunia x hybrida
21	G545	4382658	1.70E-38	Lycopersicon esculentum
21	G545	8486215	8.70E-38	Euphorbia esula
21	G545	7322653	6.80E-37	Lycopersicon hirsutum
21	G545	7785845	1.10E-33	Lotus japonicus
23	G561	2995461	5.60E-86	Sinapis alba
23	G561	633153	6.50E-83	Brassica napus

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
23	G561	1033058	5.90E-65	Raphanus sativus
23	G561	2815304	2.10E-35	Spinacia oleracea
23	G561	1498300	1.60E-34	Petroselinum crispum
23	G561	169958	8.10E-32	Glycine max
23	G561	5381310	2.20E-30	Catharanthus roseus
23	G561	1155053	9.70E-28	Phaseolus vulgaris
23	G561	728627	1.90E-27	Nicotiana tabacum
23	G561	7565950	1.40E-21	Medicago truncatula
25	G664	1167483	4.90E-81	Lycopersicon esculentum
25	G664	7765706	6.30E-69	Medicago truncatula
25	G664	19052	9.30E-68	Hordeum vulgare
25	G664	7626566	4.00E-67	Gossypium arboreum
25	G664	5050757	2.60E-66	Gossypium hirsutum
25	G664	6850206	6.90E-66	Oryza sativa
25	G664	6667606	2.20E-63	Glycine max
25	G664	517492	9.30E-62	Zea mays
25	G664	9302672	1.50E-59	Sorghum bicolor
25	G664	5860031	9.20E-58	Pinus taeda
27	G682	309571	4.40E-08	Zea mays
27	G682	4396287	1.10E-05	Glycine max
27	G682	3857004	0.00051	Populus tremula x Populus tremuloides
27	G682	9410205	0.00085	Triticum aestivum
27	G682	8382118	0.0079	Gossypium arboreum
27	G682	2428139	0.017	Oryza sativa
27	G682	7339148	0.13	Lycopersicon esculentum
27	G682	9302672	0.32	Sorghum bicolor
27	G682	5048991	0.39	Gossypium hirsutum
27	G682	6555777	0.46	Pinus taeda
29	G911	4090113	6.10E-51	Brassica napus
29	G911	5893315	7.70E-25	Lycopersicon esculentum
29	G911	5048452	3.10E-23	Gossypium hirsutum
29	G911	9440241	1.90E-21	Glycine max
29	G911	6917169	1.80E-11	Lycopersicon pennellii
29	G911	9297970	3.20E-11	Sorghum bicolor
29	G911	7137594	4.90E-11	Zea mays
29	G911	9278447	4.60E-10	Lotus japonicus
29	G911	7560271	7.20E-10	Medicago truncatula
29	G911	5043346	4.50E-09	Sorghum halepense
31	G964	7624806	3.30E-72	Gossypium arboreum
31	G964	1234899	9.10E-66	Glycine max
31	G964	1149534	1.50E-61	Pimpinella brachycarpa
31	G964	8919872	3.40E-51	Capsella rubella
31	G964	992597	6.70E-51	Lycopersicon esculentum
31	G964	1235564	1.50E-38	Oryza sativa
31	G964	6605613	3.00E-32	Medicago truncatula
31	G964	1032371	4.50E-28	Helianthus annuus
31	G964	3868846	2.80E-25	Ceratopteris richardii
31	G964	8088109	6.40E-22	Sorghum bicolor
33	G394	8670502	7.90E-59	Glycine max
33	G394	3171738	2.00E-54	Craterostigma plantagineum
33	G394	1032371	1.10E-50	Helianthus annuus
33	G394	7624806	4.30E-47	Gossypium arboreum
33	G394	1160483	2.10E-46	Pimpinella brachycarpa

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
33	G394	3868846	4.20E-45	Ceratopteris richardii
33	G394	992597	1.10E-44	Lycopersicon esculentum
33	G394	7558511	1.50E-44	Medicago truncatula
33	G394	8099247	6.20E-43	Oryza sativa
33	G394	8919872	1.20E-40	Capsella rubella
35	G489	6534956	4.40E-62	Lycopersicon esculentum
35	G489	9055852	2.60E-60	Medicago truncatula
35	G489	8382393	6.20E-51	Gossypium arboreum
35	G489	8789169	2.10E-50	Citrus x paradisi
35	G489	9252957	1.50E-47	Solanum tuberosum
35	G489	6918056	4.70E-47	Lycopersicon pennellii
35	G489	7590809	1.00E-46	Glycine max
35	G489	5257255	8.60E-43	Oryza sativa
35	G489	4152190	3.20E-41	Zea mays
35	G489	6069260	2.10E-39	Ceratodon purpureus
37	G463	6527230	4.90E-36	Lycopersicon esculentum
37	G463	9305572	5.50E-36	Sorghum bicolor
37	G463	3760881	1.20E-31	Oryza sativa
37	G463	6604917	1.30E-23	Medicago truncatula
37	G463	5058123	2.50E-21	Glycine max
37	G463	5044476	1.10E-19	Gossypium hirsutum
37	G463	9412603	1.70E-17	Triticum aestivum
37	G463	9419394	6.00E-17	Hordeum vulgare
37	G463	7624108	6.20E-17	Gossypium arboreum
37	G463	8547152	3.20E-16	Nicotiana tabacum
39	G767	5510359	2.80E-76	Glycine max
39	G767	7643155	4.20E-74	Medicago truncatula
39	G767	6977319	1.10E-72	Lycopersicon esculentum
39	G767	6730939	4.20E-68	Oryza sativa
39	G767	7502501	2.00E-67	Gossypium arboreum
39	G767	9302206	3.10E-65	Sorghum bicolor
39	G767	4218534	4.30E-51	Triticum sp.
39	G767	6732157	4.30E-51	Triticum monococcum
39	G767	9412602	6.90E-47	Triticum aestivum
39	G767	8329134	1.30E-46	Mesembryanthemum crystallinum
41	G765	4384535	3.10E-56	Lycopersicon esculentum
41	G765	6454868	8.50E-56	Glycine max
41	G765	1279639	4.30E-53	Petunia x hybrida
41	G765	4977542	2.00E-51	Oryza sativa
41	G765	4218536	2.00E-50	Triticum sp.
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41	G765	5049217	6.90E-50	Gossypium hirsutum
41	G765	9361647	4.50E-49	Triticum aestivum
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43	G197	7626566	2.40E-73	Gossypium arboreum
43	G197	7765706	1.50E-63	Medicago truncatula
43	G197	19052	8.90E-63	Hordeum vulgare
43	G197	5050757	1.60E-62	Gossypium hirsutum
43	G197	6850206	1.10E-61	Oryza sativa
43	G197	6667606	1.70E-61	Glycine max
43	G197	517492	7.60E-59	Zea mays

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
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43	G197	9302672	3.80E-55	Sorghum bicolor
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45	G255	7626566	6.40E-71	Gossypium arboreum
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45	G255	6850206	1.10E-61	Oryza sativa
45	G255	517492	3.50E-59	Zea mays
45	G255	9302672	1.60E-56	Sorghum bicolor
45	G255	7721017	2.60E-55	Lotus japonicus
47	G1113	4090113	2.30E-36	Brassica napus
47	G1113	5048452	6.80E-12	Gossypium hirsutum
47	G1113	5893315	9.50E-11	Lycopersicon esculentum
47	G1113	9440241	7.70E-09	Glycine max
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49	G398	1149534	6.20E-63	Pimpinella brachycarpa
49	G398	8919872	2.60E-47	Capsella rubella
49	G398	992597	1.10E-39	Lycopersicon esculentum
49	G398	1235564	7.70E-39	Oryza sativa
49	G398	6605613	1.70E-33	Medicago truncatula
49	G398	8088109	3.60E-33	Sorghum bicolor
49	G398	3868846	1.60E-32	Ceratopteris richardii
49	G398	3171738	1.00E-27	Craterostigma plantagineum
51	G395	992597	5.30E-51	Lycopersicon esculentum
51	G395	7624806	2.00E-50	Gossypium arboreum
51	G395	1234899	1.50E-49	Glycine max
51	G395	1165131	1.90E-48	Pimpinella brachycarpa
51	G395	3868846	3.40E-47	Ceratopteris richardii
51	G395	7415619	1.30E-41	Physcomitrella patens
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53	G393	7624806	1.30E-34	Gossypium arboreum
53	G393	7415619	1.00E-33	Physcomitrella patens
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53	G393	1235564	4.00E-32	Oryza sativa
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Riechmann, Jose Luis
Heard, Jacqueline
Ratcliffe, Oliver
Reuber, Lynne
Keddie, James

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Thr His Leu Lys Lys Lys Leu Val Met Met Lys Phe Gln Asn Gly Ile
110 115 120 125
atc aac gaa aac aaa acc aat ctg gca aca gat att tcg tct tgt aat 734
Ile Asn Glu Asn Lys Thr Asn Leu Ala Thr Asp Ile Ser Ser Cys Asn

MBI16 Sequence Listing.ST25

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aat aac aac aat gga tgt aat cac aac aaa agg acc acc aac aaa ggc			782
Asn Asn Asn Asn Gly Cys Asn His Asn Lys Arg Thr Thr Asn Lys Gly			
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Gln Trp Glu Lys Lys Leu Gln Thr Asp Ile Asn Met Ala Lys Gln Ala			
160	165	170	
tta ttc caa gcc ttg tca ctt gac caa cca tct tca ttg atc cct ccc			878
Leu Phe Gln Ala Leu Ser Leu Asp Gln Pro Ser Ser Leu Ile Pro Pro			
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Asp Pro Asp Ser Pro Lys Pro His His His Ser Thr Thr Thr Tyr Ala			
190	195	200	205
tca agc aca gat aac atc tct aaa tta ctc cag aac tgg aca agc tca			974
Ser Ser Thr Asp Asn Ile Ser Lys Leu Leu Gln Asn Trp Thr Ser Ser			
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Ser Ser Ser Lys Pro Asn Thr Ser Ser Val Ser Asn Asn Arg Ser Ser			
225	230	235	
agc ccc ggt gaa gga gga ctt ttt gat cat cac tct ttg ttc tca tcg			1070
Ser Pro Gly Glu Gly Gly Leu Phe Asp His His Ser Leu Phe Ser Ser			
240	245	250	
aat tca gaa tct gga tca gtt gat gag aag ctg aat ttg atg tcc gag			1118
Asn Ser Glu Ser Gly Ser Val Asp Glu Lys Leu Asn Leu Met Ser Glu			
255	260	265	
aca agc atg ttc aaa ggt gag agc aag cca gac ata gac atg gaa gct			1166
Thr Ser Met Phe Lys Gly Glu Ser Lys Pro Asp Ile Asp Met Glu Ala			
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aca cct act act act act act act act gat gat caa ggc tcg ttg tca			1214
Thr Pro Thr Thr Thr Thr Thr Thr Thr Asp Asp Gln Gly Ser Leu Ser			
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ttg atc gag aaa tgg ttg ttt gat gat caa ggc ttg gtt cag tgt gat			1262
Leu Ile Glu Lys Trp Leu Phe Asp Asp Gln Gly Leu Val Gln Cys Asp			
305	310	315	
gat agt caa gaa gat ctc atc gac gtg tct tta gag gag tta aaa taa			1310
Asp Ser Gln Glu Asp Leu Ile Asp Val Ser Leu Glu Glu Leu Lys			
320	325	330	
tgataacaac agtcaagatt tgttctataa gaaaataaaa cgtatagaac aacgataaag			1370
ctagctaggt ttattaattt ttctttcttt tgtcttttct ctatgatctt tagttacatt			1430
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MBI16 Sequence Listing.ST25
40 45

35

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 Ile Lys Arg Gly Asn Phe Thr Gln Pro Glu Glu Lys Met Ile Ile His
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 Leu Gln Ala Leu Leu Gly Asn Arg Trp Ala Ala Ile Ala Ser Tyr Leu
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 Pro Gln Arg Thr Asp Asn Asp Ile Lys Asn Tyr Trp Asn Thr His Leu
 100 105 110
 Lys Lys Lys Leu Val Met Met Lys Phe Gln Asn Gly Ile Ile Asn Glu
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 Asn Lys Thr Asn Leu Ala Thr Asp, Ile Ser Ser Cys Asn Asn Asn Asn
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 Asn Gly Cys Asn His Asn Lys Arg Thr Thr Asn Lys Gly Gln Trp Glu
 145 150 155 160
 Lys Lys Leu Gln Thr Asp Ile Asn Met Ala Lys Gln Ala Leu Phe Gln
 165 170 175
 Ala Leu Ser Leu Asp Gln Pro Ser Ser Leu Ile Pro Pro Asp Pro Asp
 180 185 190
 Ser Pro Lys Pro His His His Ser Thr Thr Thr Tyr Ala Ser Ser Thr
 195 200 205
 Asp Asn Ile Ser Lys Leu Leu Gln Asn Trp Thr Ser Ser Ser Ser Ser
 210 215 220
 Lys Pro Asn Thr Ser Ser Val Ser Asn Asn Arg Ser Ser Ser Pro Gly
 225 230 235 240
 Glu Gly Gly Leu Phe Asp His His Ser Leu Phe Ser Ser Asn Ser Glu
 245 250 255
 Ser Gly Ser Val Asp Glu Lys Leu Asn Leu Met Ser Glu Thr Ser Met
 260 265 270
 Phe Lys Gly Glu Ser Lys Pro Asp Ile Asp Met Glu Ala Thr Pro Thr
 275 280 285
 Thr Thr Thr Thr Thr Thr Asp Asp Gln Gly Ser Leu Ser Leu Ile Glu
 290 295 300
 Lys Trp Leu Phe Asp Asp Gln Gly Leu Val Gln Cys Asp Asp Ser Gln
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MBI16 Sequence Listing.ST25

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MBI16 Sequence Listing.ST25

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gaa gtg atg atg atg aag cat aag aag aag caa aag ggt aaa caa caa Glu Val Met Met Met Lys His Lys Lys Lys Gln Lys Gly Lys Gln Gln 220 225 230 235	1085
gaa gag tgg gac aca agt cac cac agc aac aat gat caa cat gac caa Glu Glu Trp Asp Thr Ser His His Ser Asn Asn Asp Gln His Asp Gln 240 245 250	1133
tct gcg act act tct tca aag aaa cat gtt cca cca ctt cac tct ctt Ser Ala Thr Thr Ser Ser Lys Lys His Val Pro Pro Leu His Ser Leu 255 260 265	1181
gag ttc atg gaa ctt cag aaa aga aaa gcc aag ttg ctc tcc atg ctc Glu Phe Met Glu Leu Gln Lys Arg Lys Ala Lys Leu Leu Ser Met Leu 270 275 280	1229
gaa gag ctt aaa aga aga tat gga cat tac cga gag caa atg aga gtt Glu Glu Leu Lys Arg Arg Tyr Gly His Tyr Arg Glu Gln Met Arg Val 285 290 295	1277
gcg gcg gca gcc ttt gaa gcg gcg gtt gga cta gga ggg gca gag ata Ala Ala Ala Ala Phe Glu Ala Ala Val Gly Leu Gly Gly Ala Glu Ile 300 305 310 315	1325
tac act gcg tta gcg tca agg gca atg tca aga cac ttt cgg tgt tta Tyr Thr Ala Leu Ala Ser Arg Ala Met Ser Arg His Phe Arg Cys Leu 320 325 330	1373
aaa gac gga ctt gtg gga cag att caa gca aca agt caa gct ttg gga Lys Asp Gly Leu Val Gly Gln Ile Gln Ala Thr Ser Gln Ala Leu Gly 335 340 345	1421
gag aga gaa gag gat aat cgt gcg gtt tct att gca gca cgt gga gaa Glu Arg Glu Glu Asp Asn Arg Ala Val Ser Ile Ala Ala Arg Gly Glu 350 355 360	1469
act cca cgg ttg aga ttg ctc gat caa gct ttg cgg caa cag aaa tcg Thr Pro Arg Leu Arg Leu Leu Asp Gln Ala Leu Arg Gln Gln Lys Ser 365 370 375	1517
tat cgc caa atg act ctt gtt gac gct cat cct tgg cgt cca caa cgc Tyr Arg Gln Met Thr Leu Val Asp Ala His Pro Trp Arg Pro Gln Arg 380 385 390 395	1565
ggc ttg cct gaa cgc gca gtc aca acg ttg aga gct tgg ctc ttt gaa Gly Leu Pro Glu Arg Ala Val Thr Thr Leu Arg Ala Trp Leu Phe Glu 400 405 410	1613
cac ttt ctt cac cca tat ccg agc gat gtt gat aag cat ata ttg gcc His Phe Leu His Pro Tyr Pro Ser Asp Val Asp Lys His Ile Leu Ala 415 420 425	1661
cga caa act ggt tta tca aga agt cag gta tca aat tgg ttt att aat Arg Gln Thr Gly Leu Ser Arg Ser Gln Val Ser Asn Trp Phe Ile Asn 430 435 440	1709
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gaa aca aga agt gaa caa atg gag att aca aac ccg atg atg atc gat Glu Thr Arg Ser Glu Gln Met Glu Ile Thr Asn Pro Met Met Ile Asp 460 465 470 475	1805
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MBI16 Sequence Listing.ST25

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His Gly Thr Met Ser Leu Gly Ser Thr Phe Asp Phe Ser Leu Tyr Gly			
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aac caa gct gtg aca tac gct ggt gaa gga ggg cca cgt ggt gac gtt			1997
Asn Gln Ala Val Thr Tyr Ala Gly Glu Gly Gly Pro Arg Gly Asp Val			
525	530	535	
tcc ttg acg ctt ggg tta caa cgt aac gat ggt aac ggt ggt gtg agt			2045
Ser Leu Thr Leu Gly Leu Gln Arg Asn Asp Gly Asn Gly Gly Val Ser			
540	545	550	
tta gcg ttg tct cca gtg acg gct caa ggt ggc caa ctt ttc tac ggt			2093
Leu Ala Leu Ser Pro Val Thr Ala Gln Gly Gly Gln Leu Phe Tyr Gly			
560	565	570	
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Arg Asp His Ile Glu Glu Gly Pro Val Gln Tyr Ser Ala Ser Met Leu			
575	580	585	
gat gat gat caa gtt cag aat ttg cct tat agg aat ttg atg gga gct			2189
Asp Asp Asp Gln Val Gln Asn Leu Pro Tyr Arg Asn Leu Met Gly Ala			
590	595	600	
caa tta ctt cat gat att gtt tga gattaaaaga ttaggaccaa agttatcgat			2243
Gln Leu Leu His Asp Ile Val			
605	610		
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Gln Asn Pro Thr Asp His His His Tyr Asn His Gln Ile Phe Gly Ser			
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Arg Met Thr Ser Gly Ser Asp His His His His His His Gln Thr Ser			
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Gly Gly Thr Asp Gln Asn Gln Leu Leu Glu Asp Ser Ser Ser Ala Met			
85	90	95	
Arg Leu Cys Asn Val Asn Asn Asp Phe Pro Ser Glu Val Asn Asp Glu			
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115	120	125	

MBI16 Sequence Listing.ST25

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 Gln Gln Gln Gln Gly Tyr Ser Gly Asn Lys Ser Thr Gln His Gln Asn
 145 150 155 160
 Leu Gln His Thr Gln Met Met Met Met Met Met Asn Ser His His Gln
 165 170 175
 Asn Asn Asn Asn Asn Asn His Gln His His Asn His His Gln Phe Gln
 180 185 190
 Ile Gly Ser Ser Lys Tyr Leu Ser Pro Ala Gln Glu Leu Leu Ser Glu
 195 200 205
 Phe Cys Ser Leu Gly Val Lys Glu Ser Asp Glu Glu Val Met Met Met
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 Lys His Lys Lys Lys Gln Lys Gly Lys Gln Gln Glu Glu Trp Asp Thr
 225 230 235 240
 Ser His His Ser Asn Asn Asp Gln His Asp Gln Ser Ala Thr Thr Ser
 245 250 255
 Ser Lys Lys His Val Pro Pro Leu His Ser Leu Glu Phe Met Glu Leu
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 Gln Lys Arg Lys Ala Lys Leu Leu Ser Met Leu Glu Glu Leu Lys Arg
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 Arg Tyr Gly His Tyr Arg Glu Gln Met Arg Val Ala Ala Ala Ala Phe
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 Glu Ala Ala Val Gly Leu Gly Gly Ala Glu Ile Tyr Thr Ala Leu Ala
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 Ser Arg Ala Met Ser Arg His Phe Arg Cys Leu Lys Asp Gly Leu Val
 325 330 335
 Gly Gln Ile Gln Ala Thr Ser Gln Ala Leu Gly Glu Arg Glu Glu Asp
 340 345 350
 Asn Arg Ala Val Ser Ile Ala Ala Arg Gly Glu Thr Pro Arg Leu Arg
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 Leu Leu Asp Gln Ala Leu Arg Gln Gln Lys Ser Tyr Arg Gln Met Thr
 370 375 380
 Leu Val Asp Ala His Pro Trp Arg Pro Gln Arg Gly Leu Pro Glu Arg
 385 390 395 400
 Ala Val Thr Thr Leu Arg Ala Trp Leu Phe Glu His Phe Leu His Pro
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 Tyr Pro Ser Asp Val Asp Lys His Ile Leu Ala Arg Gln Thr Gly Leu
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MBI16 Sequence Listing.ST25

Ser Arg Ser Gln Val Ser Asn Trp Phe Ile Asn Ala Arg Val Arg Leu
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Trp Lys Pro Met Ile Glu Glu Met Tyr Cys Glu Glu Thr Arg Ser Glu
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Asn Pro Thr Ser Lys Ser Gly His Asn Ser Thr His Gly Thr Met Ser
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Leu Gly Ser Thr Phe Asp Phe Ser Leu Tyr Gly Asn Gln Ala Val Thr
515 520 525

Tyr Ala Gly Glu Gly Gly Pro Arg Gly Asp Val Ser Leu Thr Leu Gly
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Leu Gln Arg Asn Asp Gly Asn Gly Gly Val Ser Leu Ala Leu Ser Pro
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Val Thr Ala Gln Gly Gly Gln Leu Phe Tyr Gly Arg Asp His Ile Glu
565 570 575

Glu Gly Pro Val Gln Tyr Ser Ala Ser Met Leu Asp Asp Asp Gln Val
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Ile Val
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Glu Leu Glu Val Gly Lys Ser Asn Leu Pro Ala Glu Ser Glu Leu Glu
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Leu Gly Leu Gly Leu Ser Leu Gly Gly Ala Trp Lys Glu Arg Gly
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MBI16 Sequence Listing.ST25

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55	60 65	
gtg gta gga tgg cca cca att ggg tta cac agg atg aac agt ttg gtt		295
Val Val Gly Trp Pro Pro Ile Gly Leu His Arg Met Asn Ser Leu Val		
70	75 80 85	
aat aac caa gct atg aag gca gca aga gcg gaa gaa gga gac ggg gag		343
Asn Asn Gln Ala Met Lys Ala Ala Arg Ala Glu Glu Gly Asp Gly Glu		
	90 95 100	
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Lys Lys Val Val Lys Asn Gly Glu Leu Lys Asp Val Ser Met Lys Val		
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Asn Pro Lys Val Gln Gly Leu Gly Phe Val Lys Val Asn Met Asp Gly		
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Val Gly Ile Gly Arg Lys Val Asp Met Arg Ala His Ser Ser Tyr Glu		
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aac ttg gct cag acg ctt gag gaa atg ttc ttt gga atg aca ggt act		535
Asn Leu Ala Gln Thr Leu Glu Glu Met Phe Phe Gly Met Thr Gly Thr		
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Thr Cys Arg Glu Thr Val Lys Pro Leu Arg Leu Leu Asp Gly Ser Ser		
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Asp Phe Val Leu Thr Tyr Glu Asp Lys Gly Ile Gly Cys Leu Leu Glu		
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 Trp Lys Glu Arg Gly Arg Ile Leu Thr Ala Lys Asp Phe Pro Ser Val		
	35 40 45	

MBI16 Sequence Listing.ST25

Gly Ser Lys Arg Ser Ala Glu Ser Ser Ser His Gln Gly Ala Ser Pro
50 55 60

Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Leu His Arg
65 70 75 80

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85 90 95

Glu Gly Asp Gly Glu Lys Lys Val Val Lys Asn Gly Glu Leu Lys Asp
100 105 110

Val Ser Met Lys Val Asn Pro Lys Val Gln Gly Leu Gly Phe Val Lys
115 120 125

Val Asn Met Asp Gly Val Gly Ile Gly Arg Lys Val Asp Met Arg Ala
130 135 140

His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu Glu Met Phe Phe
145 150 155 160

Gly Met Thr Gly Thr Thr Cys Arg Glu Thr Val Lys Pro Leu Arg Leu
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Gly Cys Leu Leu Glu Met Phe His Gly Glu Cys Leu Ser Thr Arg
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aatcattgat ggaaatgatt tgaaaaaaga gtaaagtta tttttttatt ccttgtaatt 180
ttcagaa atg ggg gat tcc gac agg gat tcc ggt gga ggg caa aac ggg 229
Met Gly Asp Ser Asp Arg Asp Ser Gly Gly Gly Gln Asn Gly 10
aac aac cag aac gga cag tcc tcc ttg tct cca aga gag caa gac agg 277
Asn Asn Gln Asn Gly Gln Ser Ser Leu Ser Pro Arg Glu Gln Asp Arg 30
15 20 25
ttc ttg ccg atc gct aac gtc agc cgg atc atg aag aag gcc ttg ccc 325
Phe Leu Pro Ile Ala Asn Val Ser Arg Ile Met Lys Lys Ala Leu Pro 45
35 40 45
gcc aac gcc aag atc tct aaa gat gcc aaa gag acg atg cag gag tgt 373
Ala Asn Ala Lys Ile Ser Lys Asp Ala Lys Glu Thr Met Gln Glu Cys 60
50 55 60
gtc tcc gag ttc atc agc ttc gtc acc gga gaa gca tct gat aag tgt 421

MBI16 Sequence Listing.ST25

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Gln	Lys	Glu	Lys	Arg	Lys	Thr	Ile	Asn	Gly	Asp	Asp	Leu	Leu	Trp	Ala	
	80					85					90					
atg	act	act	cta	ggg	ttt	gag	gat	tat	ggt	gag	cca	ttg	aaa	gtt	tac	517
Met	Thr	Thr	Leu	Gly	Phe	Glu	Asp	Tyr	Val	Glu	Pro	Leu	Lys	Val	Tyr	
	95				100					105					110	
ttg	cag	agg	ttt	agg	gag	atc	gaa	ggg	gag	agg	act	gga	cta	ggg	agg	565
Leu	Gln	Arg	Phe	Arg	Glu	Ile	Glu	Gly	Glu	Arg	Thr	Gly	Leu	Gly	Arg	
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cca	cag	act	ggg	ggg	gag	gtc	gga	gag	cat	cag	aga	gat	gct	gtc	gga	613
Pro	Gln	Thr	Gly	Gly	Glu	Val	Gly	Glu	His	Gln	Arg	Asp	Ala	Val	Gly	
			130				135						140			
gat	ggc	ggg	ggg	ttc	tac	ggg	ggg	ggg	ggg	ggg	atg	cag	tat	cac	caa	661
Asp	Gly	Gly	Gly	Phe	Tyr	Gly	Gly	Gly	Gly	Gly	Met	Gln	Tyr	His	Gln	
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cat	cat	cag	ttt	ctt	cac	cag	cag	aac	cat	atg	tat	gga	gcc	aca	ggg	709
His	His	Gln	Phe	Leu	His	Gln	Gln	Asn	His	Met	Tyr	Gly	Ala	Thr	Gly	
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Gly	Gly	Ser	Asp	Ser	Gly	Gly	Gly	Ala	Ala	Ser	Gly	Arg	Thr	Arg	Thr	
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gtata	acac	at	gcct	at	tttt	acg	accc	ata	taagg	tat	ct	atcat	gt	gat	agaacgaaca	870
ttgg	gt	ttgg	tgat	gtaaaa	tcag	at	gtgc	atta	agggtt	tag	at	tttt	tg	ggct	gtgtaa	930
aaga	agat	ca	agtg	tgct	ttt	gttg	gaca	at	aggatt	cact	aac	gaat	ctg	cttc	attgga	990
tctt	gtat	gt	aactaa	agcc	attg	tatt	ga	atgcaa	atgt	tttc	attt	gg	gatg	ctttaa		1050
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		20						25					30		
Pro	Ile	Ala	Asn	Val	Ser	Arg	Ile	Met	Lys	Lys	Ala	Leu	Pro	Ala	Asn
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Ala	Lys	Ile	Ser	Lys	Asp	Ala	Lys	Glu	Thr	Met	Gln	Glu	Cys	Val	Ser
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Glu	Phe	Ile	Ser	Phe	Val	Thr	Gly	Glu	Ala	Ser	Asp	Lys	Cys	Gln	Lys
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Glu	Lys	Arg	Lys	Thr	Ile	Asn	Gly	Asp	Asp	Leu	Leu	Trp	Ala	Met	Thr
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MBI16 Sequence Listing.ST25

Thr Leu Gly Phe Glu Asp Tyr Val Glu Pro Leu Lys Val Tyr Leu Gln
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Arg Phe Arg Glu Ile Glu Gly Glu Arg Thr Gly Leu Gly Arg Pro Gln
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 130 135 140

Gly Gly Phe Tyr Gly Gly Gly Gly Met Gln Tyr His Gln His His
 145 150 155 160

Gln Phe Leu His Gln Gln Asn His Met Tyr Gly Ala Thr Gly Gly Gly
 165 170 175

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 cgcgtccgaa ttgattagga taggatcagg atcatcctca acaacctcct cctaattcct 180
 cctccattca tagtaacaat aatattaaga aagagggtaa act atg tca gaa tta 235
 Met Ser Glu Leu
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 Leu Gln Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu
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 Val Met His Tyr Leu Cys Arg Lys Cys Ala Ser Gln Ser Ile Ala Val
 25 30 35
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 Pro Ile Ile Ala Glu Ile Asp Leu Tyr Lys Tyr Asp Pro Trp Glu Leu
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 Pro Gly Leu Ala Leu Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro
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 Arg Asp Arg Lys Tyr Pro Asn Gly Ser Arg Pro Asn Arg Ser Ala Gly
 70 75 80
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 Ser Gly Tyr Trp Lys Ala Thr Gly Ala Asp Lys Pro Ile Gly Leu Pro
 85 90 95 100
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 Lys Pro Val Gly Ile Lys Lys Ala Leu Val Phe Tyr Ala Gly Lys Ala
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MBI16 Sequence Listing.ST25

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Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys Lys Gly Ala Thr Glu Arg
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cgg gga cca cgg cct ccg gtt gtt tac ggc gac gaa atc atg gag gag 763
Arg Gly Pro Pro Pro Val Val Tyr Gly Asp Glu Ile Met Glu Glu
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Lys Pro Lys Val Thr Glu Met Val Met Pro Pro Pro Pro Gln Gln Thr
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agt gag ttc gcg tat ttc gac acg tcg gat tcg gtg ccg aag ctg cat 859
Ser Glu Phe Ala Tyr Phe Asp Thr Ser Asp Ser Val Pro Lys Leu His
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Thr Thr Asp Ser Ser Cys Ser Glu Gln Val Val Ser Pro Glu Phe Thr
215 220 225

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Ser Glu Val Gln Ser Glu Pro Lys Trp Lys Asp Trp Ser Ala Val Ser
230 235 240

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acc gtg gat aac gcg ttt gga gga gga ggg agt agt aat cag atg ttt 1051
Thr Val Asp Asn Ala Phe Gly Gly Gly Gly Ser Ser Asn Gln Met Phe
265 270 275

ccg cta cag gat atg ttc atg tac atg cag aag cct tac tag 1093
Pro Leu Gln Asp Met Phe Met Tyr Met Gln Lys Pro Tyr
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35 40 45

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MBI16 Sequence Listing.ST25

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85 90 95

Ile Gly Leu Pro Lys Pro Val Gly Ile Lys Lys Ala Leu Val Phe Tyr
100 105 110

Ala Gly Lys Ala Pro Lys Gly Glu Lys Thr Asn Trp Ile Met His Glu
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Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys Lys Asn Ser
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Leu Arg Leu Asp Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys Lys Gly
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Ala Thr Glu Arg Arg Gly Pro Pro Pro Pro Val Val Tyr Gly Asp Glu
165 170 175

Ile Met Glu Glu Lys Pro Lys Val Thr Glu Met Val Met Pro Pro Pro
180 185 190

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195 200 205

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210 215 220

Pro Glu Phe Thr Ser Glu Val Gln Ser Glu Pro Lys Trp Lys Asp Trp
225 230 235 240

Ser Ala Val Ser Asn Asp Asn Asn Asn Thr Leu Asp Phe Gly Phe Asn
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Met Ala Val Val Val Glu Glu Gly Val Val Leu Asn His Gly Gly Glu
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Glu Leu Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu
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Glu Ile Ile Thr Cys Tyr Leu Lys Glu Lys Val Leu Asn Ser Arg Phe
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Thr Ala Val Ala Met Gly Glu Ala Asp Leu Asn Lys Cys Glu Pro Trp
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Asp Leu Pro Lys Arg Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe
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Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala
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Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe
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Lys Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr
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Tyr Arg Leu Glu Gly Lys Tyr Ser Tyr Tyr Asn Leu Pro Lys Ser Ala
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Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Asn Pro Ser
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Met Asp Ser Leu Glu Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu
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ccc cat cat ttc aat tct tac caa tca atc ttt aac cac cag gtt ttt      948
Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe
245 250 255
ggg tct gct tcg ggc tct acg tac aac aac aac aac gag atg atc aag      996
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Asp Val Asn Ala Asn Met Thr Thr Thr Thr Glu Val Ser Ser Gly Pro	
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Val Met Lys Gln Glu Met Gly Met Met Gly Met Val Asn Gly Ser Lys	
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Ser Tyr Glu Asp Leu Cys Asp Leu Arg Gly Asp Leu Trp Asp Phe	
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Asp Leu Pro Lys Arg Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe	
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Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe	
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Lys Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr	
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Arg Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met His Glu	
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Tyr Arg Leu Glu Gly Lys Tyr Ser Tyr Tyr Asn Leu Pro Lys Ser Ala	
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MBI16 Sequence Listing.ST25

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195 200 205

Pro Pro Leu Ile Asp Pro Ser Phe Met Ser Gln Thr Glu Gln Pro Asn
210 215 220

Phe Lys Pro Ile Asn Pro Pro Thr Tyr Asp Ile Ser Ser Pro Ile Gln
225 230 235 240

Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe
245 250 255

Gly Ser Ala Ser Gly Ser Thr Tyr Asn Asn Asn Asn Glu Met Ile Lys
260 265 270

Met Glu Gln Ser Leu Val Ser Val Ser Gln Glu Thr Cys Leu Ser Ser
275 280 285

Asp Val Asn Ala Asn Met Thr Thr Thr Thr Glu Val Ser Ser Gly Pro
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Ala Leu Glu Ala Leu Thr Ser Pro Arg Leu Ala Ser Pro Ile Pro Pro
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Leu Phe Glu Asp Ser Ser Val Phe His Gly Val Glu His Trp Thr Lys
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ggc aag cga tct aag aga tca aga tcc gat ttc cac cac caa aac ctc 201
Gly Lys Arg Ser Lys Arg Ser Arg Ser Asp Phe His His Gln Asn Leu
35 40 45

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MBI16 Sequence Listing.ST25

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130 135 140 145	
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150 155 160	
aac aac aac atc aac act agt agc gtg tcc aac tcc gaa ggt gcg ggg Asn Asn Asn Ile Asn Thr Ser Ser Val Ser Asn Ser Glu Gly Ala Gly	585
165 170 175	
tcc act agc cac gtt agc agt agc cac cgt ggg ttt gac ctc aac atc Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn Ile	633
180 185 190	
cct ccg atc cct gaa ttc tcg atg gtc aac gga gac gac gaa gtc atg Pro Pro Ile Pro Glu Phe Ser Met Val Asn Gly Asp Asp Glu Val Met	681
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210 215 220 225	
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Gln Leu	
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Asp Asn Arg Gln Pro Pro Pro Pro Ala Val Glu Lys Leu Ser Tyr	
65 70 75 80	

MBI16 Sequence Listing.ST25

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115 120 125

Thr Thr Gly Ser Gly Lys Ser His Val Cys Thr Ile Cys Asn Lys Ser
130 135 140

Phe Pro Ser Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu
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Gly Asn Asn Asn Ile Asn Thr Ser Ser Val Ser Asn Ser Glu Gly Ala
165 170 175

Gly Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn
180 185 190

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Thr Asn Asn Ser Asp Lys Pro Ser Gln Ala Ala Ala Pro Glu Gln Ser 25
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Asn Val His Val Tyr His His Asp Trp Ala Ala Met Gln Ala Tyr Tyr 30 35 40
ggg cct aga gtt ggt ata cct caa tat tac aac tca aat ttg gcg cct 256
Gly Pro Arg Val Gly Ile Pro Gln Tyr Trp Asn Ser Asn Leu Ala Pro 45 50 55
ggt cat gct cca ccg cct tat atg tgg gcg tct cca tcg cca atg atg 304
Gly His Ala Pro Pro Pro Tyr Met Trp Ala Ser Pro Ser Pro Met Met 60 65 70

MBI16 Sequence Listing.ST25

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MBI16 Sequence Listing.ST25

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Gln Tyr Tyr Asn Ser Asn Leu Ala Pro Gly His Ala Pro Pro Pro Tyr
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Met Trp Ala Ser Pro Ser Pro Met Met Ala Pro Tyr Gly Ala Pro Tyr
 65 70 75 80

Pro Pro Phe Cys Pro Pro Gly Gly Val Tyr Ala His Pro Gly Val Gln
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Met Gly Ser Gln Pro Gln Gly Pro Val Ser Gln Ser Ala Ser Gly Val
 100 105 110

Thr Thr Pro Leu Thr Ile Asp Ala Pro Ala Asn Ser Ala Gly Asn Ser
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Asp His Gly Phe Met Lys Lys Leu Lys Glu Phe Asp Gly Leu Ala Met
 130 135 140

Ser Ile Ser Asn Asn Lys Val Gly Ser Ala Glu His Ser Ser Ser Glu
 145 150 155 160

His Arg Ser Ser Gln Ser Ser Glu Asn Asp Gly Ser Ser Asn Gly Ser
 165 170 175

Asp Gly Asn Thr Thr Gly Gly Glu Gln Ser Arg Arg Lys Arg Arg Gln
 180 185 190

Gln Arg Ser Pro Ser Thr Gly Glu Arg Pro Ser Ser Gln Asn Ser Leu
 195 200 205

Pro Leu Arg Gly Glu Asn Glu Lys Pro Asp Val Thr Met Gly Thr Pro
 210 215 220

Val Met Pro Thr Ala Met Ser Phe Gln Asn Ser Ala Gly Met Asn Gly
 225 230 235 240

MBI16 Sequence Listing.ST25

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260 265 270

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275 280 285

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290 295 300

Leu Arg Leu Glu Asn Glu Ala Ile Leu Asp Gln Leu Lys Ala Gln Ala
305 310 315 320

Thr Gly Lys Thr Glu Asn Leu Ile Ser Arg Val Asp Lys Asn Asn Ser
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<223> G664

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Met Gly Arg Ser
1
ccg tgc tgt gag aaa gct cac aca aac aaa gga gca tgg acg aaa gaa 163
Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala Trp Thr Lys Glu
5 10 15 20
gag gac gag agg ctc gtc gcc tac att aaa gct cat gga gaa ggc tgc 211
Glu Asp Glu Arg Leu Val Ala Tyr Ile Lys Ala His Gly Glu Gly Cys
25 30 35
tgg aga tct ctc ccc aaa gcc gcc gga ctt ctt cgc tgt ggc aag agc 259
Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser
40 45 50
tgc cgt ctc cgg tgg atc aac tat ctc cgg cct gac ctt aag cgt gga 307
Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly
55 60 65
aac ttc acc gag gaa gaa gac gaa ctc atc atc aag ctc cat agc ctt 355
Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu
70 75 80
ctt ggc aac aaa tgg tgc ctt att gcc ggg aga tta ccg gga aga aca 403
Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu Pro Gly Arg Thr
85 90 95 100
gat aac gag ata aag aac tat tgg aac acg cat ata cga aga aag ctt 451

MBI16 Sequence Listing.ST25

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Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile Arg Arg Lys Leu
105 110 115

ata aac aga ggg att gat cca acg agt cat aga cca atc caa gaa tca 499
Ile Asn Arg Gly Ile Asp Pro Thr Ser His Arg Pro Ile Gln Glu Ser
120 125 130

tca gct tct caa gat tct aaa cct aca caa cta gaa cca gtt acg agt 547
Ser Ala Ser Gln Asp Ser Lys Pro Thr Gln Leu Glu Pro Val Thr Ser
135 140 145

aat acc att aat atc tca ttc act tct gct cca aag gtc gaa acg ttc 595
Asn Thr Ile Asn Ile Ser Phe Thr Ser Ala Pro Lys Val Glu Thr Phe
150 155 160

cat gaa agt ata agc ttt ccg gga aaa tca gag aaa atc tca atg ctt 643
His Glu Ser Ile Ser Phe Pro Gly Lys Ser Glu Lys Ile Ser Met Leu
165 170 175

acg ttc aaa gaa gaa aaa gat gag tgc cca gtt caa gaa aag ttc cca 691
Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gln Glu Lys Phe Pro
185 190 195

gat ttg aat ctt gag ctc aga atc agt ctt cct gat gat gtt gat cgt 739
Asp Leu Asn Leu Glu Leu Arg Ile Ser Leu Pro Asp Asp Val Asp Arg
200 205 210

ctt caa ggg cat gga aag tca aca acg cca cgt tgt ttc aag tgc agc 787
Leu Gln Gly His Gly Lys Ser Thr Thr Pro Arg Cys Phe Lys Cys Ser
215 220 225

tta ggg atg ata aac ggc atg gag tgc aga tgc gga aga atg aga tgc 835
Leu Gly Met Ile Asn Gly Met Glu Cys Arg Cys Gly Arg Met Arg Cys
230 235 240

gat gta gtc gga ggt agc agc aag ggg agt gac atg agc aat gga ttt 883
Asp Val Val Gly Gly Ser Ser Lys Gly Ser Asp Met Ser Asn Gly Phe
245 250 255

gat ttt tta ggg ttg gca aag aaa gag acc act tct ctt ttg ggc ttt 931
Asp Phe Leu Gly Leu Ala Lys Lys Glu Thr Thr Ser Leu Leu Gly Phe
265 270 275

cga agc ttg gag atg aaa taa tattgtcaaa ttttaggcgt aactgtacaa 982
Arg Ser Leu Glu Met Lys
280

aacttttgcc tagataattt gaaagtatat cttcaacttg tatgagaaat ttaactggtg 1042
aattataata tatagaattt gttttttaaa aaaaaaaaaa aaaaaa 1087

<210> 26
<211> 282
<212> PRT
<213> Arabidopsis thaliana

<400> 26
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Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ala Tyr Ile Lys Ala His
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Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
35 40 45

Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
50 55 60

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MBI16 Sequence Listing.ST25

Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys
 65 70 75 80
 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu
 85 90 95
 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile
 100 105 110
 Arg Arg Lys Leu Ile Asn Arg Gly Ile Asp Pro Thr Ser His Arg Pro
 115 120 125
 Ile Gln Glu Ser Ser Ala Ser Gln Asp Ser Lys Pro Thr Gln Leu Glu
 130 135 140
 Pro Val Thr Ser Asn Thr Ile Asn Ile Ser Phe Thr Ser Ala Pro Lys
 145 150 155 160
 Val Glu Thr Phe His Glu Ser Ile Ser Phe Pro Gly Lys Ser Glu Lys
 165 170 175
 Ile Ser Met Leu Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gln
 180 185 190
 Glu Lys Phe Pro Asp Leu Asn Leu Glu Leu Arg Ile Ser Leu Pro Asp
 195 200 205
 Asp Val Asp Arg Leu Gln Gly His Gly Lys Ser Thr Thr Pro Arg Cys
 210 215 220
 Phe Lys Cys Ser Leu Gly Met Ile Asn Gly Met Glu Cys Arg Cys Gly
 225 230 235 240
 Arg Met Arg Cys Asp Val Val Gly Gly Ser Ser Lys Gly Ser Asp Met
 245 250 255
 Ser Asn Gly Phe Asp Phe Leu Gly Leu Ala Lys Lys Glu Thr Thr Ser
 260 265 270
 Leu Leu Gly Phe Arg Ser Leu Glu Met Lys
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 <212> DNA
 <213> Arabidopsis thaliana

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 <222> (1)..(228)
 <223> G682

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 act tct tct tct gaa gaa gtg agt agt ctt gag tgg gaa gtt gtg aac
 Thr Ser Ser Ser Glu Glu Val Ser Ser Leu Glu Trp Glu Val Val Asn
 20 25 30

48

96

MBI16 Sequence Listing.ST25

atg agt caa gaa gaa gaa gat ttg gtc tct cga atg cat aag ctt gtc 144
Met Ser Gln Glu Glu Glu Asp Leu Val Ser Arg Met His Lys Leu Val
35 40 45

ggg gac agg tgg gaa ctg ata gct ggg agg atc cca gga aga acc gct 192
Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala
50 55 60

gga gaa att gag agg ttt tgg gtc atg aaa aat tga 228
Gly Glu Ile Glu Arg Phe Trp Val Met Lys Asn
65 70 75

<210> 28
<211> 75
<212> PRT
<213> Arabidopsis thaliana

<400> 28

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1 5 10 15

Thr Ser Ser Ser Glu Glu Val Ser Ser Leu Glu Trp Glu Val Val Asn
20 25 30

Met Ser Gln Glu Glu Glu Asp Leu Val Ser Arg Met His Lys Leu Val
35 40 45

Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala
50 55 60

Gly Glu Ile Glu Arg Phe Trp Val Met Lys Asn
65 70 75

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<212> DNA
<213> Arabidopsis thaliana

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<222> (1)..(480)
<223> G911

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ata tta aag ata ctt tac gtc atc ggt ttc ttt aga gac atg gtc gat 96
Ile Leu Lys Ile Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp
20 25 30

gct ctt tgt cct tac att ggt cta cct agt ttt cta gac cac aac gag 144
Ala Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu
35 40 45

acc tct gga ccc gat ccg acc cga cac gct ctc tct acg tca gcg agt 192
Thr Ser Gly Pro Asp Pro Thr Arg His Ala Leu Ser Thr Ser Ala Ser
50 55 60

ctt gct aac gag ttg atc ccg gtg gtt ccg ttc tcg gat ctt ccg acc 240
Leu Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Pro Thr
65 70 75 80

gat ccg gaa gat tgt tgt acg gtt tgt ttg tca gat ttt gag tcc gac 288
Asp Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Glu Ser Asp

MB116 Sequence Listing.ST25

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100	105	110	336
tgt tta gac cgt	tgg atc gtt gac	tac aac aag atg	aaa tgt ccg gtt
Cys Leu Asp Arg	Trp Ile Val Asp	Tyr Asn Lys Met	Lys Cys Pro Val
115	120	125	384
tgt cgg cac cgg	ttc tta ccg aaa	gaa aag tac acg	caa tgt gat tgg
Cys Arg His Arg	Phe Leu Pro Lys	Glu Lys Tyr Thr	Gln Cys Asp Trp
130	135	140	432
ggt tct ggt tca	gat tgg ttt agt	gat gaa gtg gaa	agt acc aac taa
Gly Ser Gly Ser	Asp Trp Phe Ser	Asp Glu Val Glu	Ser Thr Asn
145	150	155	480

<210> 30
 <211> 159
 <212> PRT
 <213> Arabidopsis thaliana

<400> 30

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 1 5 10 15

Ile Leu Lys Ile Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp
 20 25 30

Ala Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu
 35 40 45

Thr Ser Gly Pro Asp Pro Thr Arg His Ala Leu Ser Thr Ser Ala Ser
 50 55 60

Leu Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Pro Thr
 65 70 75 80

Asp Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Glu Ser Asp
 85 90 95

Asp Lys Val Arg Gln Leu Pro Lys Cys Gly His Val Phe His His His
 100 105 110

Cys Leu Asp Arg Trp Ile Val Asp Tyr Asn Lys Met Lys Cys Pro Val
 115 120 125

Cys Arg His Arg Phe Leu Pro Lys Glu Lys Tyr Thr Gln Cys Asp Trp
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 <222> (162)..(1013)
 <223> G964

MBI16 Sequence Listing.ST25

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ttcctgaaac tgttgagttc ttgtgaaagg aaataaaaaa c atg atg atg ggc aaa      176
                                         Met Met Met Gly Lys
                                         1           5

gaa gat cta ggt ttg agc cta agc tta ggg ttt tca caa aat cac aat      224
Glu Asp Leu Gly Leu Ser Leu Ser Leu Gly Phe Ser Gln Asn His Asn
                        10                      15          20

cct ctt cag atg aat ctg aat cct aac tct tca tta tca aac aat ctc      272
Pro Leu Gln Met Asn Leu Asn Pro Asn Ser Ser Leu Ser Asn Asn Leu
                        25                      30          35

cag aga ctc cca tgg aac caa aca ttc gat cct aca tca gat ctt cgc      320
Gln Arg Leu Pro Trp Asn Gln Thr Phe Asp Pro Thr Ser Asp Leu Arg
                        40                      45          50

aag ata gac gtg aac agt ttt cca tca acg gtt aac tgc gag gaa gac      368
Lys Ile Asp Val Asn Ser Phe Pro Ser Thr Val Asn Cys Glu Glu Asp
                        55                      60          65

aca gga gtt tcg tca cca aac agt acg atc tca agc acc att agc ggg      416
Thr Gly Val Ser Ser Pro Asn Ser Thr Ile Ser Ser Thr Ile Ser Gly
                        70                      75          80          85

aag aga agt gag aga gaa gga atc tcc gga acc ggc gtt ggc tcc ggc      464
Lys Arg Ser Glu Arg Glu Gly Ile Ser Gly Thr Gly Val Gly Ser Gly
                        90                      95          100

gac gat cac gac gag atc act ccg gat cga ggg tac tca cgt gga acc      512
Asp Asp His Asp Glu Ile Thr Pro Asp Arg Gly Tyr Ser Arg Gly Thr
                        105                      110          115

tca gat gaa gaa gaa gac ggg ggc gaa acg tcg agg aag aag ctc agg      560
Ser Asp Glu Glu Glu Asp Gly Gly Glu Thr Ser Arg Lys Lys Leu Arg
                        120                      125          130

tta tca aaa gat cag tct gct ttt ctc gaa gag act ttc aaa gaa cac      608
Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Glu Thr Phe Lys Glu His
                        135                      140          145

aac act ctc aat ccc aaa cag aag cta gct ttg gct aag aag ctg aac      656
Asn Thr Leu Asn Pro Lys Gln Lys Leu Ala Leu Ala Lys Lys Leu Asn
                        150                      155          160          165

ttg acg gca aga caa gtg gaa gtg tgg ttc caa aac aga aga gct aga      704
Leu Thr Ala Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala Arg
                        170                      175          180

acc aag tta aag caa acg gag gta gat tgc gaa tac ttg aaa cgg tgc      752
Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys Arg Cys
                        185                      190          195

gta gag aag cta acg gaa gag aac cgg aga ctt cag aaa gag gct atg      800
Val Glu Lys Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu Ala Met
                        200                      205          210

gag ctt cga act ctc aag ctg tct cca caa ttc tac ggt cag atg act      848
Glu Leu Arg Thr Leu Lys Leu Ser Pro Gln Phe Tyr Gly Gln Met Thr
                        215                      220          225

cca cca act aca ctc atc atg tgt cct tcg tgc gag cgt gta gct ggt      896
Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys Glu Arg Val Ala Gly
                        230                      235          240          245

cca tca tca tcg aac cat cac cac aat cac agg ccg gtt tcg att aac      944
Pro Ser Ser Ser Asn His His His Asn His Arg Pro Val Ser Ile Asn
                        250                      255          260

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MBI16 Sequence Listing.ST25

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 265 270 275

gcc ttg cgt cca cga tcg taa ttttagtggtg tgggggaagg gtgttttggg 1043
 Ala Leu Arg Pro Arg Ser
 280

ttttttcatt atcgttatat agtctatctg tgtgggggtca ttgtaatttt ggatgattgg 1103

ccttctcatg aactagtcac atgtatgatg caaccttaaa aatattttcaa gtagcaaaac 1163

ttaattacaa acttgctata ttaacaaaaa attatgaaaa aaaaaaaaaa aaaaaaaa 1221

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 <211> 283
 <212> PRT
 <213> Arabidopsis thaliana

<400> 32

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 20 25 30

Leu Ser Asn Asn Leu Gln Arg Leu Pro Trp Asn Gln Thr Phe Asp Pro
 35 40 45

Thr Ser Asp Leu Arg Lys Ile Asp Val Asn Ser Phe Pro Ser Thr Val
 50 55 60

Asn Cys Glu Glu Asp Thr Gly Val Ser Ser Pro Asn Ser Thr Ile Ser
 65 70 75 80

Ser Thr Ile Ser Gly Lys Arg Ser Glu Arg Glu Gly Ile Ser Gly Thr
 85 90 95

Gly Val Gly Ser Gly Asp Asp His Asp Glu Ile Thr Pro Asp Arg Gly
 100 105 110

Tyr Ser Arg Gly Thr Ser Asp Glu Glu Glu Asp Gly Gly Glu Thr Ser
 115 120 125

Arg Lys Lys Leu Arg Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Glu
 130 135 140

Thr Phe Lys Glu His Asn Thr Leu Asn Pro Lys Gln Lys Leu Ala Leu
 145 150 155 160

Ala Lys Lys Leu Asn Leu Thr Ala Arg Gln Val Glu Val Trp Phe Gln
 165 170 175

Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu
 180 185 190

Tyr Leu Lys Arg Cys Val Glu Lys Leu Thr Glu Glu Asn Arg Arg Leu
 195 200 205

Gln Lys Glu Ala Met Glu Leu Arg Thr Leu Lys Leu Ser Pro Gln Phe

MBI16 Sequence Listing.ST25

210 215 220

Tyr Gly Gln Met Thr Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys
225 230 235 240

Glu Arg Val Ala Gly Pro Ser Ser Ser Asn His His His Asn His Arg
245 250 255

Pro Val Ser Ile Asn Pro Trp Ile Ala Cys Ala Gly Gln Val Ala His
260 265 270

Gly Leu Asn Phe Glu Ala Leu Arg Pro Arg Ser
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<211> 1249
<212> DNA
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<222> (82)..(918)
<223> G394

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Met Gly Leu Asp Asp Ser Cys Asn Thr Gly
1 5 10
ctt gtt ctt ggt tta ggc ctc tca cca acg cct aat aat tac aat cat 159
Leu Val Leu Gly Leu Gly Leu Ser Pro Thr Pro Asn Asn Tyr Asn His
15 20 25
gcc atc aag aaa tct tcc tcc act gtg gac cat cgt ttc atc agg ctc 207
Ala Ile Lys Lys Ser Ser Ser Thr Val Asp His Arg Phe Ile Arg Leu
30 35 40
gat ccg tcg ttg act cta agc cta tcc ggt gag agc tac aag atc aag 255
Asp Pro Ser Leu Thr Leu Ser Leu Ser Gly Glu Ser Tyr Lys Ile Lys
45 50 55
act ggt gcc ggc gcc ggc gac caa att tgc cgg cag acc tcg tcc cac 303
Thr Gly Ala Gly Ala Gly Asp Gln Ile Cys Arg Gln Thr Ser Ser His
60 65 70
agc ggc atc tca tct ttc tcg agc gga agg gta aag aga gaa aga gaa 351
Ser Gly Ile Ser Ser Phe Ser Ser Gly Arg Val Lys Arg Glu Arg Glu
75 80 85 90
atc tcc ggc ggc gat gga gaa gaa gag gcg gag gag acg acg gag aga 399
Ile Ser Gly Gly Asp Gly Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg
95 100 105
gtg gtg tgt tcg aga gtg agt gat gat cat gac gat gaa gaa ggt gtt 447
Val Val Cys Ser Arg Val Ser Asp Asp His Asp Asp Glu Glu Gly Val
110 115 120
agt gct cgt aaa aag ctt aga ctc act aaa caa caa tct gct ctt ctc 495
Ser Ala Arg Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu
125 130 135
gaa gat aac ttc aaa ctt cat agc acc ctt aat ccc aag caa aaa caa 543
Glu Asp Asn Phe Lys Leu His Ser Thr Leu Asn Pro Lys Gln Lys Gln
140 145 150
gct ctt gcg aga cag ctg aat cta agg cct aga caa gtt gaa gtg tgg 591
Ala Leu Ala Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp

WO 01/36598

MBI16 Sequence Listing.ST25

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ttc caa aac agg aga gct aga aca aaa cta aag caa aca gaa gtg gat				639
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	175	180	185	
tgt gag ttt ttg aag aaa tgt tgc gag act tta acg gat gag aat aga				687
Cys Glu Phe Leu Lys Lys Cys Cys Glu Thr Leu Thr Asp Glu Asn Arg				
	190	195	200	
agg ctt caa aaa gag ctt caa gac ctt aag gct tta aaa ttg tct caa				735
Arg Leu Gln Lys Glu Leu Gln Asp Leu Lys Ala Leu Lys Leu Ser Gln				
	205	210	215	
ccg ttt tac atg cac atg ccg gcg gcg act ttg act atg tgc cct tct				783
Pro Phe Tyr Met His Met Pro Ala Ala Thr Leu Thr Met Cys Pro Ser				
	220	225	230	
tgt gag aga ctc ggc ggt ggt ggt gtc gga gga gat acg acg gcg gtt				831
Cys Glu Arg Leu Gly Gly Gly Gly Val Gly Gly Asp Thr Thr Ala Val				
	235	240	245	250
gat gaa gaa acg gcg aaa gga gct ttc tcc atc gtc aca aag cct cgt				879
Asp Glu Glu Thr Ala Lys Gly Ala Phe Ser Ile Val Thr Lys Pro Arg				
	255	260	265	
ttc tat aac cct ttc act aat cct tct gca gca tgt tag ttacttatta				928
Phe Tyr Asn Pro Phe Thr Asn Pro Ser Ala Ala Cys				
	270	275		
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agaagataaaa tcccaggga aaaatattac gttgaaattg gggggaaatg gggatatagtc				1048
tttatagata agactcttca acgattccac tttatttttc ggtgggattg ttggttgatg				1108
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 <212> PRT
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<400> 34

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 20 25 30

Ser Thr Val Asp His Arg Phe Ile Arg Leu Asp Pro Ser Leu Thr Leu
 35 40 45

Ser Leu Ser Gly Glu Ser Tyr Lys Ile Lys Thr Gly Ala Gly Ala Gly
 50 55 60

Asp Gln Ile Cys Arg Gln Thr Ser Ser His Ser Gly Ile Ser Ser Phe
 65 70 75 80

Ser Ser Gly Arg Val Lys Arg Glu Arg Glu Ile Ser Gly Gly Asp Gly
 85 90 95

Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg Val Val Cys Ser Arg Val

MBI16 Sequence Listing.ST25

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 130 135 140
 His Ser Thr Leu Asn Pro Lys Gln Lys Gln Ala Leu Ala Arg Gln Leu
 145 150 155 160
 Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala
 165 170 175
 Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe Leu Lys Lys
 180 185 190
 Cys Cys Glu Thr Leu Thr Asp Glu Asn Arg Arg Leu Gln Lys Glu Leu
 195 200 205
 Gln Asp Leu Lys Ala Leu Lys Leu Ser Gln Pro Phe Tyr Met His Met
 210 215 220
 Pro Ala Ala Thr Leu Thr Met Cys Pro Ser Cys Glu Arg Leu Gly Gly
 225 230 235 240
 Gly Gly Val Gly Gly Asp Thr Thr Ala Val Asp Glu Glu Thr Ala Lys
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 260 265 270
 Asn Pro Ser Ala Ala Cys
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 <210> 35
 <211> 1147
 <212> DNA
 <213> Arabidopsis thaliana
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 <222> (33)..(695)
 <223> G489
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 Tyr Gln Thr Asn Pro Met Ser Thr Thr Ala Ala Thr Val Ala Gly Gly
 10 15 20
 gcg gca caa cca ggc cag ctg gcg ttc cac cag atc cat cag cag cag 149
 Ala Ala Gln Pro Gly Gln Leu Ala Phe His Gln Ile His Gln Gln Gln
 25 30 35
 cag cag caa cag ctg gca cag cag ctt caa gca ttt tgg gag aac caa 197
 Gln Gln Gln Gln Leu Ala Gln Gln Leu Gln Ala Phe Trp Glu Asn Gln
 40 45 50 55

MBI16 Sequence Listing.ST25

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atc tcg gct gag gcg ccg gtc gtg ttt gca agg gcc tgt gag atg ttc Ile Ser Ala Glu Ala Pro Val Val Phe Ala Arg Ala Cys Glu Met Phe 90 95 100	341
atc ctg gag ctg aca ctc agg tcg tgg aac cac act gag gag aat aag Ile Leu Glu Leu Thr Leu Arg Ser Trp Asn His Thr Glu Glu Asn Lys 105 110 115	389
agg cgg acg ttg cag aag aac gat att gct gct gct gtg act aga acc Arg Arg Thr Leu Gln Lys Asn Asp Ile Ala Ala Ala Val Thr Arg Thr 120 125 130 135	437
gat att ttt gat ttc ctt gtg gat att gtt ccc cgg gag gat ctc cga Asp Ile Phe Asp Phe Leu Val Asp Ile Val Pro Arg Glu Asp Leu Arg 140 145 150	485
gat gaa gtc ttg gga agt att ccg agg ggc act gtc ccg gaa gct gct Asp Glu Val Leu Gly Ser Ile Pro Arg Gly Thr Val Pro Glu Ala Ala 155 160 165	533
gct gct ggt tac ccg tat gga tac ttg cct gca gga act gct cca ata Ala Ala Gly Tyr Pro Tyr Gly Tyr Leu Pro Ala Gly Thr Ala Pro Ile 170 175 180	581
gga aat ccg gga atg gtt atg ggt aat ccc ggt ggt gcg tat cca cct Gly Asn Pro Gly Met Val Met Gly Asn Pro Gly Gly Ala Tyr Pro Pro 185 190 195	629
aat cct tat atg ggt caa cca atg tgg caa caa cag gca cct gac caa Asn Pro Tyr Met Gly Gln Pro Met Trp Gln Gln Gln Ala Pro Asp Gln 200 205 210 215	677
cct gac cag gaa aat tag caagaaactg tgagtcttcc agcttcgcgg Pro Asp Gln Glu Asn 220	725
ccgctctaga caggcctcgt accggatcct ctagctagag ctttcgttcg tatcatcggt	785
ttcgacaacg ttcgtcaagt tcaatgcac agtttcattg cgcacacacc agaatcctac	845
tgagtttgag tattatggca ttgggaaaac tgtttttctt gtccatttgt tgtgcttgta	905
atttactgtg ttttttattc ggttttcgct atcgaaactgt gaaatggaaa tggatggaga	965
agagttaatg aatgatatgg ccttttgttc attctcaaat taatattatt tggtttttct	1025
cttatttgtg gggatgaatt tgaaattata agagatatgc aaacattttg tttgagtaaa	1085
atgtgcaaat cgtggcctct aatgacccga agttaatatg aggagtaaaa cacttgtagg	1145
tg	1147
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Ala Ala Thr Val Ala Gly Gly Ala Ala Gln Pro Gly Gln Leu Ala Phe 20 25 30	

MBI16 Sequence Listing.ST25

His Gln Ile His Gln Gln Gln Gln Gln Gln Gln Leu Ala Gln Gln Leu
35 40 45

Gln Ala Phe Trp Glu Asn Gln Phe Lys Glu Ile Glu Lys Thr Thr Asp
50 55 60

Phe Lys Asn His Ser Leu Pro Leu Ala Arg Ile Lys Lys Ile Met Lys
65 70 75 80

Ala Asp Glu Asp Val Arg Met Ile Ser Ala Glu Ala Pro Val Val Phe
85 90 95

Ala Arg Ala Cys Glu Met Phe Ile Leu Glu Leu Thr Leu Arg Ser Trp
100 105 110

Asn His Thr Glu Glu Asn Lys Arg Arg Thr Leu Gln Lys Asn Asp Ile
115 120 125

Ala Ala Ala Val Thr Arg Thr Asp Ile Phe Asp Phe Leu Val Asp Ile
130 135 140

Val Pro Arg Glu Asp Leu Arg Asp Glu Val Leu Gly Ser Ile Pro Arg
145 150 155 160

Gly Thr Val Pro Glu Ala Ala Ala Ala Gly Tyr Pro Tyr Gly Tyr Leu
165 170 175

Pro Ala Gly Thr Ala Pro Ile Gly Asn Pro Gly Met Val Met Gly Asn
180 185 190

Pro Gly Gly Ala Tyr Pro Pro Asn Pro Tyr Met Gly Gln Pro Met Trp
195 200 205

Gln Gln Gln Ala Pro Asp Gln Pro Asp Gln Glu Asn
210 215 220

<210> 37
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<222> (217)..(957)
<223> G463

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ctttcaaaag actctttctt tcttttggat tgattttgga ttctagggct ctctttcttt 180
tagtgggttt ttgttggtgt tggttggtgc tctctg atg att act gaa ctt gag 234
Met Ile Thr Glu Leu Glu
1 5
atg ggg aaa ggt gag agt gag ctt gag ctt ggt cta ggg ctg agt ctt 282
Met Gly Lys Gly Glu Ser Glu Leu Glu Leu Gly Leu Gly Leu Ser Leu
10 15 20

MBI16 Sequence Listing.ST25

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tgg gga gag cgt gga agg ctt ttg acg gct aag gat ttt cct tct gtt Trp Gly Glu Arg Gly Arg Leu Leu Thr Ala Lys Asp Phe Pro Ser Val 40 45 50	378
ggt tct aaa cgt gct gct gat tct gct tct cat gct ggt tca tct cct Gly Ser Lys Arg Ala Ala Asp Ser Ala Ser His Ala Gly Ser Ser Pro 55 60 65 70	426
cct cgt tca agt caa gtt gtt gga tgg cct cct ata ggg tca cac agg Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Ser His Arg 75 80 85	474
atg aac agt ttg gtt aat aac caa gct aca aag tca gca aga gaa gaa Met Asn Ser Leu Val Asn Asn Gln Ala Thr Lys Ser Ala Arg Glu Glu 90 95 100	522
gaa gaa gct ggt aag aag aaa gtg aaa gat gat gaa cct aaa gat gtg Glu Glu Ala Gly Lys Lys Lys Val Lys Asp Asp Glu Pro Lys Asp Val 105 110 115	570
aca aag aaa gtg aat ggg aaa gta caa gtt gga ttt att aag gtg aac Thr Lys Lys Val Asn Gly Lys Val Gln Val Gly Phe Ile Lys Val Asn 120 125 130	618
atg gat gga gtt gct ata gga aga aaa gtg gat ttg aat gct cat tct Met Asp Gly Val Ala Ile Gly Arg Lys Val Asp Leu Asn Ala His Ser 135 140 145 150	666
tct tac gag aat ttg gcg caa aca ttg gaa gat atg ttc ttt cgc act Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu Asp Met Phe Phe Arg Thr 155 160 165	714
aat ccg ggt act gtc ggg tta acc agt cag ttc act aaa ccg ttg agg Asn Pro Gly Thr Val Gly Leu Thr Ser Gln Phe Thr Lys Pro Leu Arg 170 175 180	762
ctt tta gat gga tcg tct gag ttt gta ctt act tat gaa gat aag gaa Leu Leu Asp Gly Ser Ser Glu Phe Val Leu Thr Tyr Glu Asp Lys Glu 185 190 195	810
gga gat tgg atg ctt gtt ggt gat gtt cca tgg aga atg ttc atc aac Gly Asp Trp Met Leu Val Gly Asp Val Pro Trp Arg Met Phe Ile Asn 200 205 210	858
tcg gtg aaa agg cta cgt gtg atg aaa acc tct gaa gct aat gga ctc Ser Val Lys Arg Leu Arg Val Met Lys Thr Ser Glu Ala Asn Gly Leu 215 220 225 230	906
gct gca cga aat caa gaa cca aac gag aga cag cga aag cag ccg gtt Ala Ala Arg Asn Gln Glu Pro Asn Glu Arg Gln Arg Lys Gln Pro Val 235 240 245	954
tag atctcttttc gacgttacgg tggtagcgg tttatatattt ggggttttgc	1007
aagtcctgaga tacttctgaa gcaagcataa gctagattga tcttatatcc agtttgtgta	1067
ttttcttggt tcttataatg gtttttactg gttttcttta gttttttttt ttgctgtctt	1127
ttaatttttcg gttgcgattt cactatatac tatggatgga agagaatgct ctttatatct	1187
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tgacgtagcc tcgag	1262

<210> 38
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MBI16 Sequence Listing.ST25

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Gly Leu Gly Leu Ser Leu Gly Gly Gly Thr Ala Ala Lys Ile Gly Lys
 20 25 30

Ser Gly Gly Gly Gly Ala Trp Gly Glu Arg Gly Arg Leu Leu Thr Ala
 35 40 45

Lys Asp Phe Pro Ser Val Gly Ser Lys Arg Ala Ala Asp Ser Ala Ser
 50 55 60

His Ala Gly Ser Ser Pro Pro Arg Ser Ser Gln Val Val Gly Trp Pro
 65 70 75 80

Pro Ile Gly Ser His Arg Met Asn Ser Leu Val Asn Asn Gln Ala Thr
 85 90 95

Lys Ser Ala Arg Glu Glu Glu Glu Ala Gly Lys Lys Lys Val Lys Asp
 100 105 110

Asp Glu Pro Lys Asp Val Thr Lys Lys Val Asn Gly Lys Val Gln Val
 115 120 125

Gly Phe Ile Lys Val Asn Met Asp Gly Val Ala Ile Gly Arg Lys Val
 130 135 140

Asp Leu Asn Ala His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu
 145 150 155 160

Asp Met Phe Phe Arg Thr Asn Pro Gly Thr Val Gly Leu Thr Ser Gln
 165 170 175

Phe Thr Lys Pro Leu Arg Leu Leu Asp Gly Ser Ser Glu Phe Val Leu
 180 185 190

Thr Tyr Glu Asp Lys Glu Gly Asp Trp Met Leu Val Gly Asp Val Pro
 195 200 205

Trp Arg Met Phe Ile Asn Ser Val Lys Arg Leu Arg Val Met Lys Thr
 210 215 220

Ser Glu Ala Asn Gly Leu Ala Ala Arg Asn Gln Glu Pro Asn Glu Arg
 225 230 235 240

Gln Arg Lys Gln Pro Val
 245

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 <211> 905
 <212> DNA
 <213> Arabidopsis thaliana

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 <222> (76)..(837)
 <223> G767

MBI16 Sequence Listing.ST25

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                  Met Met Lys Ser Gly Ala Asp Leu Gln Phe Pro Pro
                  1          5          10
gga ttt aga ttt cat cct acg gat gag gag cta gtc ctc atg tat ctc      159
Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Leu Met Tyr Leu
                  15          20          25
tgt cgt aaa tgc gcg tcg cag ccg atc cct gct ccg att atc acc gaa      207
Cys Arg Lys Cys Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu
                  30          35          40
ctc gat ttg tac cga tat gat cct tgg gac ctt ccc gac atg gct ttg      255
Leu Asp Leu Tyr Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu
                  45          50          55
tac ggt gaa aag gag tgg tat ttt ttc tca cca aga gat cga aag tat      303
Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr
                  65          70          75
cca aac ggt tca aga ccc aac cgt gca gct ggt act gga tat tgg aaa      351
Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys
                  80          85          90
gct acc gga gct gat aaa cca ata ggt cgt cct aaa ccg gtt ggt att      399
Ala Thr Gly Ala Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile
                  95          100          105
aag aag gct cta gtg ttt tac tcg gga aaa cct cca aat gga gag aaa      447
Lys Lys Ala Leu Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys
                  110          115          120
acc aat tgg att atg cac gaa tac cgg ctc gct gac gtt gac cgg tcg      495
Thr Asn Trp Ile Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser
                  125          130          135
gtt cgt aag aaa aac agt cta aga ttg gac gat tgg gta ttg tgt cgt      543
Val Arg Lys Lys Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg
                  145          150          155
ata tat aac aag aaa ggt gtc atc gag aag cga cga agc gat atc gag      591
Ile Tyr Asn Lys Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu
                  160          165          170
gac ggg tta aag cct gtg act gac acg tgt cca ccg gaa tct gtg gcg      639
Asp Gly Leu Lys Pro Val Thr Asp Thr Cys Pro Pro Glu Ser Val Ala
                  175          180          185
aga ttg atc tcc ggc tcg gag caa gcg gtg tca ccg gaa ttc acg tgt      687
Arg Leu Ile Ser Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys
                  190          195          200
agc aac ggt cgg ttg agt aat gcc ctt gat ttt ccg ttt aat tac gta      735
Ser Asn Gly Arg Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val
                  205          210          215
gat gcc atc gcc gat aac gag att gtg tca cgg cta ttg ggc ggg aat      783
Asp Ala Ile Ala Asp Asn Glu Ile Val Ser Arg Leu Leu Gly Gly Asn
                  225          230          235
cag atg tgg tcg acg acg ctt gat cca ctt gtg gtt agg cag gga act      831
Gln Met Trp Ser Thr Thr Leu Asp Pro Leu Val Val Arg Gln Gly Thr
                  240          245          250
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Phe
ttttgcacg tgctcgggc      905

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MBI16 Sequence Listing.ST25

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<213> Arabidopsis thaliana

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20          25          30

Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu Leu Asp Leu Tyr
35          40          45

Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu Tyr Gly Glu Lys
50          55          60

Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr Pro Asn Gly Ser
65          70          75          80

Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys Ala Thr Gly Ala
85          90          95

Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile Lys Lys Ala Leu
100         105         110

Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys Thr Asn Trp Ile
115         120         125

Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys
130         135         140

Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys
145         150         155         160

Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu Asp Gly Leu Lys
165         170         175

Pro Val Thr Asp Thr Cys Pro Pro Glu Ser Val Ala Arg Leu Ile Ser
180         185         190

Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys Ser Asn Gly Arg
195         200         205

Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val Asp Ala Ile Ala
210         215         220

Asp Asn Glu Ile Val Ser Arg Leu Leu Gly Gly Asn Gln Met Trp Ser
225         230         235         240

Thr Thr Leu Asp Pro Leu Val Val Arg Gln Gly Thr Phe
245         250

<210> 41
<211> 1479
<212> DNA
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MBI16 Sequence Listing.ST25

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 <222> (192)..(962)
 <223> G765

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cgatctcctc aaaaagttat tgttttcttg aaggattttt cttgttcttg atcaagcata      180
catatatata g atg gtg gaa gaa ggc ggc gta gtt gtg aat caa gga gga      230
          Met Val Glu Glu Gly Gly Val Val Val Asn Gln Gly Gly
          1          5          10

gat cag gag gtg gtg gat ttg cct ccg ggg ttt cgg ttt cac cct act      278
Asp Gln Glu Val Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr
          15          20          25

gat gaa gag ata ata act cac tac ctc aaa gag aag gtc ttc aac atc      326
Asp Glu Glu Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile
          30          35          40          45

cga ttt acc gcg gca gcg att ggt caa gcc gac ctc aac aag aac gag      374
Arg Phe Thr Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu
          50          55          60

cca tgg gat cta cca aag att gca aag atg ggg gag aag gag ttt tac      422
Pro Trp Asp Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr
          65          70          75

ttt ttc tgc cag agg gat cgg aag tat ccg acc ggg atg agg acg aac      470
Phe Phe Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn
          80          85          90

cgt gcg acc gtg tct ggt tat tgg aag gcg acc ggg aag gac aag gag      518
Arg Ala Thr Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu
          95          100          105

atc ttt aga ggc aaa ggt tgt ctt gtt ggg atg aag aaa aca ctt gtt      566
Ile Phe Arg Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val
          110          115          120          125

ttc tat aca gga aga gct cct aaa ggt gaa aag acc aat tgg gtt atg      614
Phe Tyr Thr Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met
          130          135          140

cat gaa tat cgt ctt gat gga aaa tat tct tat cat aac ctc ccc aaa      662
His Glu Tyr Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys
          145          150          155

acc gca agg gat gaa tgg gtg gtg tgt agg gtt ttt cac aag aac gct      710
Thr Ala Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala
          160          165          170

cct agt act aca atc act act aca aaa caa ctc tca agg att gat tct      758
Pro Ser Thr Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser
          175          180          185

ctt gat aac att gat cat ctc tta gac ttc tca tct ctc cct cct ctc      806
Leu Asp Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu
          190          195          200          205

ata gat ccg ggt ttc ttg ggt caa ccc gcc caa gct tct ccg gtg ccc      854
Ile Asp Pro Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro
          210          215          220

gtc aac aac acg atc tca aac ctg tct cca cca tcc tac aac cgc acc      902
Val Asn Asn Thr Ile Ser Asn Leu Ser Pro Pro Ser Tyr Asn Arg Thr
          225          230

agt cga caa cac tta cct tcc tac cca agc tct caa ttt ccc tta cca      950

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MBI16 Sequence Listing.ST25

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 aataacaaag gtatgatcaa gttggagcat tctcttgatga gtgtgtctca agaaaccggt 1062
 ttgagttccg atgtgaacac aaccgcaacg ccagagatat cttcttatcc aatgatgatg 1122
 aatccggcaa tgatggatgg tagcaagtca gcgtgtgatg gtcttgatga cttgatcttc 1182
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 agtggcatat tgattactcg tctagtgttt ttaatcgtgt aattagttcg tatataatat 1302
 acatgtacat aagatcatta ggtttattag gcattggact ttagttcggg gattgcttac 1362
 ctagttttta gcttgagaaa aaaggctgtc attgggggtta tgtttctttg tgattaactt 1422
 gtacatatat acatttaaata taaacgtatg gtttaaatcg tttaaaaaaa aaaaaaa 1479

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 <212> PRT
 <213> Arabidopsis thaliana

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 20 25 30
 Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile Arg Phe Thr
 35 40 45
 Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu Pro Trp Asp
 50 55 60
 Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe Cys
 65 70 75 80
 Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala Thr
 85 90 95
 Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg
 100 105 110
 Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr
 115 120 125
 Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met His Glu Tyr
 130 135 140
 Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys Thr Ala Arg
 145 150 155 160
 Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala Pro Ser Thr
 165 170 175

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MBI16 Sequence Listing.ST25

Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser Leu Asp Asn
180 185 190

Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu Ile Asp Pro
195 200 205

Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro Val Asn Asn
210 215 220

Thr Ile Ser Asn Leu Ser Pro Pro Ser Tyr Asn Arg Thr Ser Arg Gln
225 230 235 240

His Leu Pro Ser Tyr Pro Ser Ser Gln Phe Pro Leu Pro Leu Gly Pro
245 250 255

<210> 43
<211> 825
<212> DNA
<213> Arabidopsis thaliana

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<223> G197

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Trp Thr Lys Glu Glu Asp Asp Lys Leu Ile Ser Tyr Ile Lys Ala His
20 25 30
ggg gaa ggt tgt tgg cgt tct ctt cct aga tcc gcc ggt ctt caa cgt 144
Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg
35 40 45
tgc gga aaa agc tgt cgt ctc cga tgg att aac tat ctc cga cct gat 192
Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
50 55 60
ctc aag agg ggt aac ttc acc ctc gaa gaa gat gat ctc atc atc aaa 240
Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Leu Ile Ile Lys
65 70 75 80
cta cat agc ctt ctc ggt aac aag tgg tct ctt att gcg acg aga tta 288
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu
85 90 95
cca gga aga aca gat aac gag att aag aat tac tgg aac aca cat gtt 336
Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val
100 105 110
aag agg aag cta tta aga aaa ggg att gat ccg gcg act cat cga cct 384
Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro
115 120 125
atc aac gag acc aaa act tct caa gat tcg tct gat tct agt aaa aca 432
Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr
130 135 140
gag gac cct ctt gtc aag att ctc tct ttt ggt cct cag ctg gag aaa 480
Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys
145 150 155 160
ata gca aat ttc ggg gac gag aga att caa aag aga gtt gag tac tca 528
Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser
165 170 175

MBI16 Sequence Listing.ST25

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Val Val Glu Glu Arg Cys Leu Asp Leu Asn Leu Glu Leu Arg Ile Ser
180 185 190

cca cca tgg caa gac aag ctc cat gat gag agg aac cta agg ttt ggg 624
Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly
195 200 205

aga gtg aag tat agg tgc agt gcg tgc cgt ttt gga ttc ggg aac ggc 672
Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly
210 215 220

aag gag tgt agc tgt aat aat gtg aaa tgt caa aca gag gac agt agt 720
Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser
225 230 235 240

agc agc agt tat tct tca acc gac att agt agt agc att ggt tat gac 768
Ser Ser Ser Tyr Ser Ser Thr Asp Ile Ser Ser Ser Ile Gly Tyr Asp
245 250 255

ttc ttg ggt cta aac aac act agg gtt ttg gat ttt agc act ttg gaa 816
Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu
260 265 270

atg aaa tga 825
Met Lys

<210> 44
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<212> PRT
<213> Arabidopsis thaliana

<400> 44

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Trp Thr Lys Glu Glu Asp Asp Lys Leu Ile Ser Tyr Ile Lys Ala His
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Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg
35 40 45

Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
50 55 60

Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys
65 70 75 80

Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu
85 90 95

Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val
100 105 110

Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro
115 120 125

Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr
130 135 140

Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys
145 150 155 160

MBI16 Sequence Listing.ST25

Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser
 165 170 175

Val Val Glu Glu Arg Cys Leu Asp Leu Asn Leu Glu Leu Arg Ile Ser
 180 185 190

Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly
 195 200 205

Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly
 210 215 220

Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser
 225 230 235 240

Ser Ser Ser Tyr Ser Ser Thr Asp Ile Ser Ser Ser Ile Gly Tyr Asp
 245 250 255

Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu
 260 265 270

Met Lys

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 <211> 918
 <212> DNA
 <213> Arabidopsis thalina

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 <222> (30)..(839)
 <223> G255

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aaa gaa cac atg aac aaa ggt gct tgg act aaa gaa gaa gat gag aga 101
 Lys Glu His Met Asn Lys Gly Ala Trp Thr Lys Glu Glu Asp Glu Arg
 10 15 20

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 Pro Arg Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser Cys Arg Leu Arg
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 Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Asn Phe Thr His
 60 65 70

gat gaa gat gaa ctt atc atc aag ctt cat agc ctc cta ggc aac aag 293
 Asp Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu Leu Gly Asn Lys
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MBI16 Sequence Listing.ST25

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 105 110 115 120
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 Ile Asp Pro Ala Thr His Arg Gly Ile Asn Glu Ala Lys Ile Ser Asp
 125 130 135
 ttg aag aaa aca aag gac caa att gta aaa gat gtt tct ttt gtg aca 485
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 140 145 150
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 Lys Phe Glu Glu Thr Asp Lys Ser Gly Asp Gln Lys Gln Asn Lys Tyr
 155 160 165
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 Ile Arg Asn Gly Leu Val Cys Lys Glu Glu Arg Val Val Val Glu Glu
 170 175 180
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 Lys Ile Gly Pro Asp Leu Asn Leu Glu Leu Arg Ile Ser Pro Pro Trp
 185 190 195 200
 caa aac cag aga gaa ata tct act tgc act gcg tcc cgt ttt tac atg 677
 Gln Asn Gln Arg Glu Ile Ser Thr Cys Thr Ala Ser Arg Phe Tyr Met
 205 210 215
 gaa aac gac atg gag tgt agt agt gaa act gtg aaa tgt caa aca gag 725
 Glu Asn Asp Met Glu Cys Ser Ser Glu Thr Val Lys Cys Gln Thr Glu
 220 225 230
 aat agt agc agc att agc tat tct tct att gat att agt agt agt aac 773
 Asn Ser Ser Ser Ile Ser Tyr Ser Ser Ile Asp Ile Ser Ser Ser Asn
 235 240 245
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 Val Gly Tyr Asp Phe Leu Gly Leu Lys Thr Arg Ile Leu Asp Phe Arg
 250 255 260
 agc ttg gaa atg aaa taa atgaatagta ttagattcct aatttgtagg 869
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 35 40 45
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 50 55 60
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 65 70 75 80
 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Ala Arg Leu
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MBI16 Sequence Listing.ST25

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 130 135 140

Val Lys Asp Val Ser Phe Val Thr Lys Phe Glu Glu Thr Asp Lys Ser
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Gly Asp Gln Lys Gln Asn Lys Tyr Ile Arg Asn Gly Leu Val Cys Lys
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Glu Glu Arg Val Val Val Glu Glu Lys Ile Gly Pro Asp Leu Asn Leu
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Glu Leu Arg Ile Ser Pro Pro Trp Gln Asn Gln Arg Glu Ile Ser Thr
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Cys Thr Ala Ser Arg Phe Tyr Met Glu Asn Asp Met Glu Cys Ser Ser
 210 215 220

Glu Thr Val Lys Cys Gln Thr Glu Asn Ser Ser Ser Ile Ser Tyr Ser
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 Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu Thr Ser Arg Ser
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MBI16 Sequence Listing.ST25

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 Leu Ile Pro Val Val Arg Phe Ser Asp Leu Leu Thr Asp Pro Glu Asp
 70 75 80
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 Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp Lys Ile Arg
 85 90 95
 cag ctg ccg aag tgt gga cac gtg ttt cat cat cgt tgt tta gac cgt 392
 Gln Leu Pro Lys Cys Gly His Val Phe His His Arg Cys Leu Asp Arg
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 Trp Ile Val Asp Cys Asn Lys Ile Thr Cys Pro Ile Cys Arg Asn Arg
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 Phe Leu Pro Glu Glu Lys Ser Thr Pro Phe Asp Trp Gly Thr Ser Asp
 135 140 145
 tgg ttt aga gat gaa gtg gag agt acc aac taa taatgatggt tttactttta 541
 Trp Phe Arg Asp Glu Val Glu Ser Thr Asn
 150 155
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 35 40 45
 Ser Arg Ser Asp Pro Thr Arg Leu Ala Leu Ser Thr Ser Ala Thr Leu
 50 55 60
 Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Leu Thr Asp
 65 70 75 80
 Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp
 85 90 95
 Lys Ile Arg Gln Leu Pro Lys Cys Gly His Val Phe His His Arg Cys
 100 105 110
 Leu Asp Arg Trp Ile Val Asp Cys Asn Lys Ile Thr Cys Pro Ile Cys
 115 120 125
 Arg Asn Arg Phe Leu Pro Glu Glu Lys Ser Thr Pro Phe Asp Trp Gly
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MBI16 Sequence Listing.ST25

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 145 150 155

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 tctcattttc taccaagaga caatatc atg atg atg ggt aaa gag gat ttg ggt 174
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 Leu Lys Pro Thr Ser Ser Pro Met Ser Asn Leu Gln Met Phe Pro Trp
 30 35 40
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 Asn Gln Thr Leu Val Ser Ser Ser Asp Gln Gln Lys Gln Gln Phe Leu
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 Arg Lys Ile Asp Val Asn Ser Leu Pro Thr Thr Val Asp Leu Glu Glu
 60 65 70
 gag aca gga gtt tcg tct cca aac agt acg atc tcg agc aca gtg agt 414
 Glu Thr Gly Val Ser Ser Pro Asn Ser Thr Ile Ser Ser Thr Val Ser
 75 80 85
 gga aag agg agg agt act gaa aga gaa ggt acc tcc ggt ggt ggt tgc 462
 Gly Lys Arg Arg Ser Thr Glu Arg Glu Gly Thr Ser Gly Gly Gly Cys
 90 95 100 105
 gga gat gac ctt gac atc act cta gat aga tct tcc tca cgt gga acc 510
 Gly Asp Asp Leu Asp Ile Thr Leu Asp Arg Ser Ser Ser Arg Gly Thr
 110 115 120
 tcc gat gaa gag gaa gat tac gga ggt gag act tgt agg aag aag ctt 558
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 125 130 135
 aga cta tcc aaa gat caa tcc gca gtt ctc gaa gac act ttc aaa gag 606
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 140 145 150
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 His Asn Thr Leu Asn Pro Lys Gln Lys Leu Ala Leu Ala Lys Lys Leu
 155 160 165
 ggt tta aca gca aga caa gtg gaa gtg tgg ttc caa aac aga aga gca 702
 Gly Leu Thr Ala Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala
 170 175 180 185
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 Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys Arg
 190 195 200
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 Cys Val Glu Lys Leu Thr Glu Glu Asn Arg Arg Leu Glu Lys Glu Ala
 205 210 215

MBI16 Sequence Listing.ST25

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agt cca ccg acc aca ctt ttg atg tgt cca tcg tgt gaa cgt gtg gcc 894
Ser Pro Pro Thr Thr Leu Met Cys Pro Ser Cys Glu Arg Val Ala
235 240 245

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250 255 260 265

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Trp Leu Gln Met Ala His Gly Ser Thr Phe Asp Val Met Arg Pro Arg
270 275 280

tct taa ctttaatgct gcttctatgg gttgtgtgtg ggtcattgta ctttttagat 1046
Ser

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50 55 60

Leu Pro Thr Thr Val Asp Leu Glu Glu Glu Thr Gly Val Ser Ser Pro
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Arg Glu Gly Thr Ser Gly Gly Gly Cys Gly Asp Asp Leu Asp Ile Thr
100 105 110

Leu Asp Arg Ser Ser Ser Arg Gly Thr Ser Asp Glu Glu Glu Asp Tyr
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145 150 155 160

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MBI16 Sequence Listing.ST25

Gln Lys Leu Ala Leu Ala Lys Lys Leu Gly Leu Thr Ala Arg Gln Val
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Glu Val Asp Cys Glu Tyr Leu Lys Arg Cys Val Glu Lys Leu Thr Glu
 195 200 205

Glu Asn Arg Arg Leu Glu Lys Glu Ala Ala Glu Leu Arg Ala Leu Lys
 210 215 220

Leu Ser Pro Arg Leu Tyr Gly Gln Met Ser Pro Pro Thr Thr Leu Leu
 225 230 235 240

Met Cys Pro Ser Cys Glu Arg Val Ala Gly Pro Ser Ser Ser Asn His
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 atg ccc tta gga gca gct acg gtt gtg gag gag gaa gag gag gag gag 167
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 Glu Ala Val Pro Ser Met Ser Val Ser Pro Pro Asp Ser Val Thr Ser
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 Ser Asn Lys Arg Asp Ile Asp Asp Glu Val Glu Arg Ser Ala Ser Arg
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 Ala Ser Asn Glu Asp Asn Asp Asp Glu Asn Gly Ser Thr Arg Lys Lys
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MBI16 Sequence Listing.ST25

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Arg Cys Cys Glu Ser Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu
      145                      150                      155                      160

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Val Lys Glu Leu Arg Thr Leu Lys Thr Ser Thr Pro Phe Tyr Met Gln
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      210                      215                      220

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Ser
      225

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50      55      60

Ala Ser Asn Glu Asp Asn Asp Asp Glu Asn Gly Ser Thr Arg Lys Lys
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Leu Arg Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Asp Ser Phe Lys
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Glu His Ser Thr Leu Asn Pro Lys Gln Lys Ile Ala Leu Ala Lys Gln
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MBI16 Sequence Listing.ST25

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Ser
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Asn Tyr Asn Ser Thr Ile Arg Gln Ser Ser Val Tyr Lys Leu Glu Pro
25 30 35
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Ser Leu Thr Leu Cys Leu Ser Gly Asp Pro Ser Val Thr Val Val Thr
40 45 50
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Ser Phe Ser Ser Gly Arg Val Val Lys Arg Glu Arg Asp Gly Gly Glu
75 80 85
gag tcg ccg gag gag gaa gag atg acg gag aga gtt ata agt gat tac 342
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90 95 100
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 135 140 145 150

cct aga caa gtt gaa gta tgg ttt caa aat aga aga gcc agg aca aag 534
 Pro Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala Arg Thr Lys
 155 160 165

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Val Tyr Lys Leu Glu Pro Ser Leu Thr Leu Cys Leu Ser Gly Asp Pro
 35 40 45

Ser Val Thr Val Val Thr Gly Ala Asp Gln Leu Cys Arg Gln Thr Ser
 50 55 60

Ser His Ser Gly Val Ser Ser Phe Ser Ser Gly Arg Val Val Lys Arg
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MBI16 Sequence Listing.ST25

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 Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe
 165 170 175
 Leu Lys Lys Cys Cys Glu Thr Leu Ala Asp Glu Asn Ile Arg Leu Gln
 180 185 190
 Lys Glu Ile Gln Glu Leu Lys Thr Leu Lys Leu Thr Gln Pro Phe Tyr
 195 200 205
 Met His Met Pro Ala Ser Thr Leu Thr Lys Cys Pro Ser Cys Glu Arg
 210 215 220
 Ile Gly Gly Gly Gly Gly Gly Asn Gly Gly Gly Gly Gly Ser Gly
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 Ala Thr Ala Val Ile Val Asp Gly Ser Thr Ala Lys Gly Ala Phe Ser
 245 250 255
 Ile Ser Ser Lys Pro His Phe Phe Asn Pro Phe Thr Asn Pro Ser Ala
 260 265 270
 Ala Cys

INTERNATIONAL SEARCH REPORT

In al application No.

PCT/US00/31458

A. CLASSIFICATION OF SUBJECT MATTER												
IPC(7) : C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, 15/74, 15/82, 15/87; C07H 21/02, 21/04; A01H 1/00, 9/00, 11/00												
US CL : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
P,X ---	Database GenEmbl on STIC, USPTO, (Arlington, VA, USA), GenBank Accession AC002388, LIN et al. 'Sequence analysis of chromosome 2 of the plant Arabidopsis thaliana,' abstract, Nature, 1999, Vol. 402, 761-768.	4-6 ----- 1-3, 7-13, 25-27										
P,Y ---	Database EST on STIC, USPTO, (Arlington, VA, USA), GenBank Accession AV552445, ASAMIZU et al. 'A large scale analysis of cDNA in Arabidopsis thaliana: generation of 12,028 non-redundant expressed sequence tags from normalized and size-selected cDNA libraries,' abstract, DNA Research, 2000, Vol. 7, 175-180.	4-6 ----- 1-3, 7-13, 25-27										
X ---	Database EST on STIC, USPTO, (Arlington, VA, USA), Genbank Accession AI997809, CHEN et al. unpublished, abstract, 08 September 1999.	4-6 ----- 1-3, 7-13, 25-27										
Y ---	Database EST on STIC, USPTO, (Arlington, VA, USA), GenBank Accession N97133, NEWMAN et al. 'Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones,' abstract, Plant Physiology, 1994, Vol. 106, 1241-1255.	4-6 ----- 1-3, 7-13, 25-27										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.												
* Special categories of cited documents: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 13 February 2001 (13.02.2001)		Date of mailing of the international search report 07 MAR 2001										
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Cynthia Collins <i>Lilla Collins for</i> Telephone No. (703) 605-1210										

INTERNATIONAL SEARCH REPORT

I application No.

PCT/US00/31458

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database EST on STIC, USPTO, (Arlington, VA, USA). GenBank Accession AA598183, NEWMAN et al. 'Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones.' abstract. Plant Physiology, 1994, Vol. 106, 1241-1255.	4-6 ----- 1-3, 7-13, 25-27
X --- Y	Database PIR_66 on STIC, USPTO, (Arlington, VA, USA), Accession T00409, ROUNSLEY et al. unpublished, abstract, 01 February 1999.	11 ----- 1-10, 12-13, 25-27
X --- Y	Database SPTREMBL_15 on STIC, USPTO, (Arlington, VA, USA), Accession 022167, ROUNSLEY et al. unpublished, abstract, 01 January 1998.	11 ----- 1-10, 12-13, 25-27
T,E	RIECHMANN et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science, 15 December 2000, Vol. 290, pages 2105-2110.	
P,A	SUNG et al. Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower buds and fruits in apple. Planta, March 2000, Vol. 210, pages 519-528.	
P,Y	RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology, October 2000, Vol. 3, pages 423-434, especially pages 427-428.	1-13, 25-27
Y	US 5,892,009 A (THOMASHOW et al.) 06 April 1999, column 14, lines 1-46.	1-3, 7-10, 12-13, 25-27
A	RATCLIFFE et al. Separation of shoot and floral identity in Arabidopsis. Development, March 1999, Vol. 126, pages 1109-1120.	
A	SUNG et al. Characterization of MdMADS2, a member of the SQUAMOSA subfamily of genes, in apple. Plant Physiology, August 1999, Vol. 120, pages 969-978.	
A	RIECHMANN et al. The AP2/EREBP family of plant transcription factors. Biol. Chem. June 1998, Vol. 379, pages 633-646.	
A	RIECHMANN et al. Determination of floral organ identity by Arabidopsis MADS domain homeotic proteins AP1, AP3, PL, and AG is independent of their DNA-binding specificity. Molecular Biology of the Cell, July 1997, Vol., pages 1243-1259.	
A	HEARD et al. Evolutionary diversity of symbiotically induced nodule MADS box genes: characterization of umhCS, a member of a novel subfamily. Molecular Plant-Microbe Interactions, July 1997, Vol. 10, No. 5, pages 665-676.	
A	RIECHMANN et al. MADS domain proteins in plant development. Biol. Chem. October 1997, Vol. 378, pages 1079-1101.	
A	RIECHMANN et al. DNA-binding properties of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA and AGAMOUS. Nucleic Acids Research, August 1996, Vol. 24, No. 16, pages 3134-3141.	
A	RIECHMANN et al. Dimerization specificity of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. Proc. Natl. Acad. Sci. USA, May 1996, Vol. 93, pages 4793-4798.	
A	HEARD et al. Symbiotic induction of a MADS-box gene during development of alfalfa root nodules. Proc. Natl. Acad. Sci. USA, June 1995, Vol. 92, pages 5273-5277.	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31458

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13, 25-27 SEQ ID NOS: 1 and 2

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

1 nat application No.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXVII, claim(s) 1-13 and 25-27, drawn to transgenic plants with modified environmental stress tolerance, polynucleotides and vectors for producing said transgenic plants, and methods of making said transgenic plants. Applicant must elect one pair of sequences (one nucleotide sequence and its corresponding amino acid translation) per Group to be examined, i.e. SEQ ID NOS: 1 and 2 in Group I, SEQ ID NOS: 3 and 4 in Group II, SEQ ID NOS: 5 and 6 in Group III, etc.

Group XXVIII, claim(s) 15-17, drawn to a method of identifying a factor that is modulated by or interacts with a polypeptide.

Group XXIX, claim(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest.

Group XXX, claim(s) 19 and 20, drawn to an integrated system, computer, or computer readable medium.

Group XXXI, claim(s) 21-23, drawn to a method of identifying a polynucleotide sequence.

The inventions listed as Groups I-XXXI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXVII are drawn to transgenic plants and methods of producing said plants with nucleic acid sequences. The methods of Groups I-XXVII differ from each other in that they are directed to plant transformation methods and transgenic plants with structurally and functionally distinct nucleic acid sequences which encode structurally and functionally different amino acid sequences. In addition, Groups XXVIII, XXIX, and XXXI are different methods from any of Groups I-XXVII in that they have different method steps and different end products, and Group XXX requires a computer system. Thus, there is no single special technical feature which links the inventions of Groups I-XXXI under PCT Rule 13.2.

Continuation of B. FIELDS SEARCHED Item 3: STN (agricola, biosis, biotechno, biotechds, biotechabs, caba, caplus, embase, medline, uspatfull, wpids, pctfull, europatfull, japio) SEARCH TERMS: inventor names, plant transcription factor, stress tolerance; STIC sequence search for SEQ ID NOS: 1 and 2